

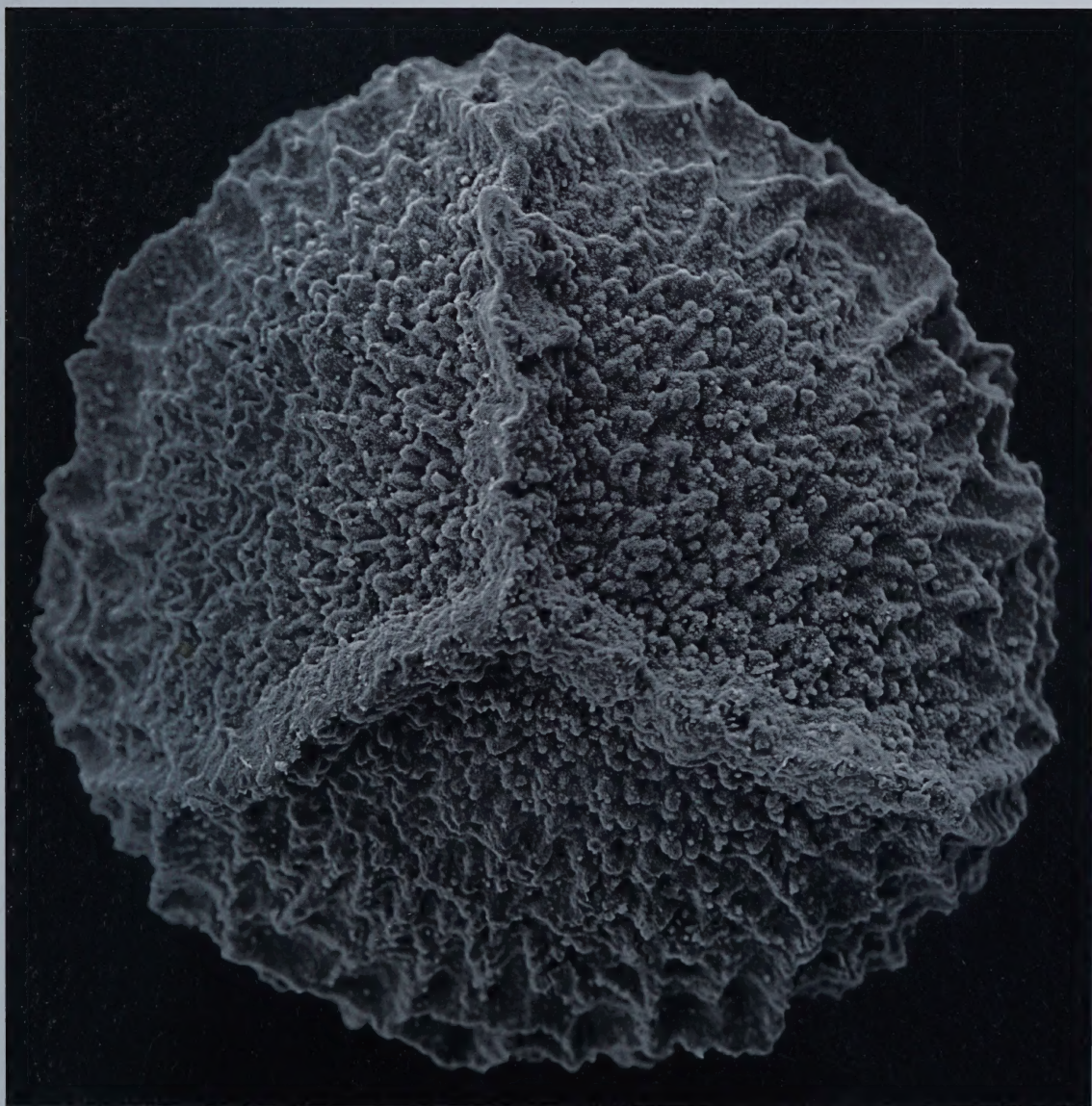
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Megaspore Investigations of *Selaginella* Species from São Paulo, Brazil

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ABSTRACT.—The lycophyte genus *Selaginella* has a worldwide distribution with ca. 800 species. The genus is heterosporous and has ornamentation on the surface of both the megaspores and the microspores. *Selaginella* megaspore ornamentation is highly variable in both its macro- and microsculpture. Megaspore ornamentation is a species-specific morphological feature that can be used reliably for specimen identification. We present the first investigation of the megaspore surface of all 19 *Selaginella* species known from the state of São Paulo, Brazil, using scanning electronic microscopy (SEM). This study also includes *Selaginella* species that are invasive in Brazil. Based on the SEM analysis and high-resolution photographs, we provide detailed megaspore descriptions and an identification key for all species from São Paulo state. We also describe the proximal and distal pole ornamentation of the megaspores as well as the form and proportions of the trilete aperture.

KEY WORDS.—heterospory, lycophytes, ornamentation, South America, SEM

The genus *Selaginella* P. Beauv. has the highest diversity within the lycophytes, and comprises ca. 800 species (Banks 2009; Frey and Engler 2009; Jermy 1990; Zhou and Zhang 2015). It occupies a worldwide distribution, but occurs mainly in the tropics and subtropics (Jermy 1990; Setyawan 2011; Tryon and Lugardon 1991). Research on *Selaginella* has increased in recent years and has included molecular phylogenetic studies (Arrigo *et al.* 2013;

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Korall and Kenrick 2002, 2004; Korall *et al.* 1999; Zhou *et al.* 2015), a completely sequenced genome (Banks *et al.* 2011), transcription factor investigations (e.g., Gramzow *et al.* 2012), growth and ontogenetic research (Schulz *et al.* 2010a, b), and descriptions of many new species (e.g., Valdespino *et al.* 2015).

The genus *Selaginella* is heterosporous, with large megaspores and small microspores. The spores are trilete and tetrahedral to globose. Megaspore surface sculpture is frequently reticulate with additional ornamentation (e.g., echinae, gemmae, or baculae). Many studies have used scanning electron microscopy (SEM) to examine megaspore surface sculpture in detail (Little *et al.* 2007; Punt *et al.* 2007; Schulz *et al.* 2010; Schulz *et al.* 2013; Zhao *et al.* 2006; Zhou and Zhang 2015). Several studies have shown that some megaspore surface characters are suitable for species identification (Blackmore 2007; Korall and Taylor 2006; Minaki 1984; Schulz *et al.* 2013; Tryon 1949; Zhou *et al.* 2015a; Zhou *et al.* 2015c), and so the megaspore surfaces of several *Selaginella* species have been investigated with respect to their exospore layers (Hellwig 1969; Morbelli 1977; Taylor 1989). The exospore exhibits specific patterns of ornamentation that may be suitable for taxon identification and delimitation at the species level (Morbelli *et al.* 2001; Tryon and Lugardon 1991; Schulz *et al.* 2013; Valdespino 1993).

Schulz *et al.* (2013) have shown that megaspore surfaces are extremely diverse within *Selaginella* subg. *Ericetorum* (Jermy 1986), and that spore surfaces display sufficient information to identify species using an identification key that includes both megaspores and microspores. Megaspore characters are useful not only for the identification of recent *Selaginella* species but also for that of extinct species; this is important since recent studies have examined both fossil *Selaginella* megaspores of small areas (Glasspool *et al.* 2000; Glasspool 2003) and extant species from large states such as Rio Grande do Sul in Brazil (Lorscheitter *et al.* 2005; Lorscheitter *et al.* 2002).

Jermy (1986) defined five subgenera within the genus *Selaginella* based on morphological features: *Selaginella*, *Ericetorum*, *Tetragonostachys*, *Heterostachys*, and *Stachygynandrum*. Current investigations, based on molecular and morphological analyses, including spore features, recognize the following six clades as subgenera within the genus *Selaginella* (Zhou *et al.* 2015a; Zhou and Zhang 2015). (1) *Selaginella* subg. *Selaginella* (Jermy 1986), including *Selaginella deflexa* and *Selaginella selaginoides*; this clade is sister to all other *Selaginella* species. (2) The *S. sanguinolenta* clade, also called *Selaginella* subg. *Boreoselaginella*, which has contiguous tubercle megaspore surfaces and is sister to all other clades except *Selaginella* subg. *Selaginella* (Zhou *et al.* 2015a); representatives of this clade were formerly included in Jermy's *Selaginella* subg. *Stachygynandrum* (1986). (3) The *Ericetorum* superclade, also called *Selaginella* subg. *Ericetorum*, which has reticulate megaspore surface sculptures (Zhou *et al.* 2015a; Zhou and Zhang 2015); within this *Ericetorum* superclade, the species in Jermy's work are further subdivided into different groups, e.g., *Ericetorum*, *Stachygynandrum*, and *Tetragonostachys*. (4) The *Rosulatae* clade, also called *Selaginella*

subg. *Pulvinella*, which exhibits coarse and spaced verrucate megaspore surfaces (Zhou *et al.* 2015a; Zhou and Zhang 2015); the species of this clade/section were assigned to the subg. *Stachygynandrum* by Jermy (1986). (5) The superclade *Heterostachys* (*Selaginella* subg. *Heterostachys*) (Zhou *et al.* 2015a; Zhou and Zhang 2015), which includes species that were included in Jermy's *Selaginella* subg. *Heterostachys* and *Selaginella* subg. *Stachygynandrum* (Jermy 1986); the representatives of this *Heterostachys* superclade have megaspore surfaces with a wide range of ornamentation, from tuberculate and verrucate through rugulate (Zhou *et al.* 2015a; Zhou and Zhang 2015). Finally, (6) the superclade *Stachygynandrum* (also called *Selaginella* subg. *Stachygynandrum*), which includes species with reticulate megaspore surfaces (Zhou *et al.* 2015a; Zhou and Zhang 2015); the representatives of this superclade/subgenus were already classified as subg. *Stachygynandrum* by Jermy (1986).

Selaginella species from tropical and subtropical South America have been the subject of several morphological investigations (Alston *et al.* 1981; Crabbe and Jermy 1973; Tryon and Tryon 1982). In the context of those studies, the Brazilian species of *Selaginella* have been examined in detail (Alston 1936), and detailed identification keys and species descriptions have been published for several states of Brazil (Assis and Labiak 2009; Góes-Neto *et al.* 2015; Hirai and Prado 2000; Hirai *et al.* 2007; Prado and Freitas 2005; Valdespino *et al.* 2015). However, vegetative characters are not sufficient to correctly identify problematic specimens, such as herbarium specimens that are incomplete or in poor condition. Furthermore, correct identification is more reliable when both vegetative and megaspore surface characters are used. Extensive recent species descriptions, for example those of Valdespino *et al.* (2015), Valdespino (2014; 2015a, 2015b), and Zhou *et al.* (2015a, 2015b), already include megaspore surface descriptions.

The purpose of our study was to investigate the megaspore surface diversity of the *Selaginella* species occurring in the Brazilian state of São Paulo. Based on our results, we generated an identification key using megaspore surface characters (as shown in high-resolution images) that allows these *Selaginella* species to be identified. We also examined our results in the context of current comprehensive investigations that have revealed several new superclades, subgenera, clades, sections, and subclades within the genus *Selaginella* (Zhou and Zhang 2015; Zhou *et al.* 2015a). Our study includes native *Selaginella* species as well as documented invasive *Selaginella* species from São Paulo (Hirai 2014; Hirai and Prado 2000).

MATERIALS AND METHODS

All known *Selaginella* species from the São Paulo state were investigated, based on the check list "Lista de Espécies da Flora do Brasil" by Hirai (2014). Specimens for SEM investigations were used from the following herbaria: F, L, SP, UPCB, and HRCB (Table 1). For each species, at least 15 megaspores from different accessions were investigated (these specimens are marked with an

TABLE 1. Taxa included in study, voucher information, references to figures, and classification after Zhou and Zhang (2015), (–) = not classified.

Species	Voucher	Figure	Subgenus	Section
<i>S. contigua</i>	Cesar 647 (HRCB)	Fig. 1 A–C	<i>Stachygynandrum</i>	–
<i>S. convoluta</i>	Coelho 83 (UPCB)	Fig. 1 D–F	<i>Pulvinella</i>	–
<i>S. decomposita</i>	Lombardi 7853 (HRCB)	Fig. 1 G–I	<i>Ericetorum</i>	<i>Articulatae</i>
<i>S. flexuosa</i>	Bauer 12 (HRCB)	Fig. 1 J–L	<i>Stachygynandrum</i>	<i>Heterophyllae</i>
<i>S. kraussiana</i>	Jallu 5300 (L)	Fig. 2 A–C	<i>Ericetorum</i>	<i>Articulatae</i>
<i>S. macrostachya</i>	Attili 6625 (HRCB)	Fig. 2 D–F	<i>Stachygynandrum</i>	–
<i>S. marginata</i>	Bauer 21 (HRCB)	Fig. 2 G–I	<i>Ericetorum</i>	<i>Articulatae</i>
<i>S. mendoncae</i>	Glaziou 12297 (F)	Fig. 2 J–L	<i>Stachygynandrum</i>	–
<i>S. microphylla</i>	Bauer 22 (HRCB)	Fig. 3 A–C	<i>Stachygynandrum</i>	<i>Heterophyllae</i>
<i>S. muscosa</i>	Bauer 15 (HRCB)	Fig. 3 D–F	<i>Heterostachys</i>	–
<i>S. pallescens</i>	Schaffer 241 (L)	Fig. 3 G–I	<i>Stachygynandrum</i>	<i>Pallescentes</i>
<i>S. plana</i>	Meer 2036 (L)	Fig. 3 J–L	<i>Heterostachys</i>	<i>Oligomacrosporangiatæ</i>
<i>S. sellowii</i>	Shepherd 5854 (F)	Fig. 4 A–C	<i>Ericetorum</i>	<i>Homeophyllae</i>
<i>S. suavis</i>	Labiak 4416 (UPCB)	Fig. 4 D–F	<i>Ericetorum</i>	<i>Articulatae</i>
<i>S. sulcata</i>	Dittrich 1022 (HRCB)	Fig. 4 G–I	<i>Ericetorum</i>	<i>Articulatae</i>
<i>S. tenuissima</i>	Pereira 350 (UPCB)	Fig. 4 J–L	<i>Ericetorum</i>	<i>Articulatae</i>
<i>S. valida</i>	Aragaki 69 (SP)	Fig. 5 A–C	<i>Stachygynandrum</i>	–
<i>S. vestiens</i>	Pereira 703 (UPCB)	Fig. 5 D–F	<i>Stachygynandrum</i>	–
<i>S. vogelii</i>	Lombardi 8410 (HRCB)	Fig. 5 G–I	<i>Heterostachys</i>	<i>Oligomacrosporangiatæ</i>

asterisk in “Additional Specimens Examined”) to detect putative species variability. Additional specimens were investigated morphologically to confirm the taxonomic classification. The dried material was fixed on aluminum stubs using a carbon tape, sputter-coated with gold for 180 s using a Sputter Coater SCD 050 (BALZERS) and studied using a DSM 950 SEM (ZEISS) at 10 kV. Images were digitally recorded using the Digital Image Processing System (DIPS; POINT ELECTRONICS).

The terms used below (e.g. baculate, clavate, echinate, scabrate, reticulate, perforate) follow the definitions by Punt *et al.* (2007), Korall and Taylor (2006), and Schulz *et al.* (2013). The reticulum forms a network of muri; if the network is connected the reticulum is “closed”, if the network is disconnected, the reticulum is “open” (Korall and Taylor 2006). The term “curvaturæ perfectæ” define a line in trilete spores extending from the extremities of the ends of the radii of the laesurae and thus delimiting the contact areas that are continuous around the proximal face. The “complex mass at the pole” defines a structure found on the pole of the spore (Punt *et al.* 2007, Korall and Taylor 2006). The term “laesurae” define the arms of a proximal fissure or scar of a spore, and “aperture” is a specialized region of the sporoderm and generally differs in ornamentation and/or in structure (Punt *et al.* 2007). The term “underlying lumina” characterizes the part of the muri that meets the enclosed space under the muri (Korall and Taylor 2006; Schulz *et al.* 2013). The megaspore

ornamentation size follows the definition by Schulz *et al.* (2013): microsculpture (descriptive terms with prefix “micro”) <5 µm and macrosculpture ≥5µm.

RESULTS

KEY TO *SELAGINELLA* SPECIES OF SÃO PAULO STATE BASED ON MEGASPORE CHARACTERS

- 1. Spore without reticulum 2
 - 2. Macrosculpture (proximal face) gemmate *S. plana*
 - 2. Macrosculpture (proximal face) verrucate *S. convoluta*
- 1. Spore with reticulum 3
 - 3. Reticulum (proximal face) absent. 4
 - 4. Macrosculpture (proximal face) baculate, reticulate *S. pallescens*
 - 4. Macrosculpture (proximal face) clavate, gemmate. 5
 - 5. Microsculpture (distal face) scabrate *S. mendoncae*
 - 5. Microsculpture (distal face) micro-echinate *S. vogelii*
 - 3. Reticulum (proximal face) present 6
 - 6. Sculpture in the center, close to the laesurae differs from the sculpture along the edge (proximal face) 7
 - 7. The height of the laesurae is descending towards to the pole *S. muscosa* Spring
 - 7. The height of the laesurae is increasing towards to the pole or has same height ... 8
 - 8. Macrosculpture (distal face) rugulate-cristate 9
 - 9. Macrosculpture (proximal face) gemmate. *S. flexuosa*
 - 9. Macrosculpture (proximal face) clavate *S. macrostachya*
 - 8. Macrosculpture (distal face) rugulate 10
 - 10. Macrosculpture (proximal face) verrucate. *S. microphylla*
 - 10. Macrosculpture (proximal face) baculate *S. vestiens*
 - 6. Sculpture in the center, close to the laesurae does not differ from the sculpture along the edge (proximal face) 11
 - 11. The height of the laesurae is descending towards to the pole *S. valida*
 - 11. The height of the laesurae is increasing towards to the pole or has same height ... 12
 - 12. Macrosculpture (distal face) reticulate 13
 - 13. Macrosculpture (proximal face) striate *S. suavis*
 - 13. Macrosculpture (proximal face) rugulate-cristate *S. decomposita*
 - 12. Macrosculpture (distal face) rugulate 14
 - 14. Macrosculpture (proximal face) rugulate-cristate *S. tenuissima*
 - 14. Macrosculpture (proximal face) striate 15
 - 15. Macrosculpture (distal face) verrucate 16
 - 16. Macrosculpture (proximal face) psilate. *S. sellowii*
 - 16. Macrosculpture (proximal face) clavate 17
 - 17. Microsculpture (proximal face) micro-rugulate *S. contigua*
 - 17. Microsculpture (proximal face) micro-clavate *S. kraussiana*
 - 15. Macrosculpture (distal face) rugulate 18
 - 18. Macrosculpture (proximal face) verrucate. *S. marginata*
 - 18. Macrosculpture (proximal face) reticulate *S. sulcata*

DESCRIPTION OF THE MEGASPORES FOR EACH SPECIES

Selaginella contigua Baker, J. Bot. 22:295. no. 162. 1884. *Lycopodioides contigua* (Baker) Kuntze, Rev. Gen. Pl. 1:826. 1891. Fig. 1 A–C.

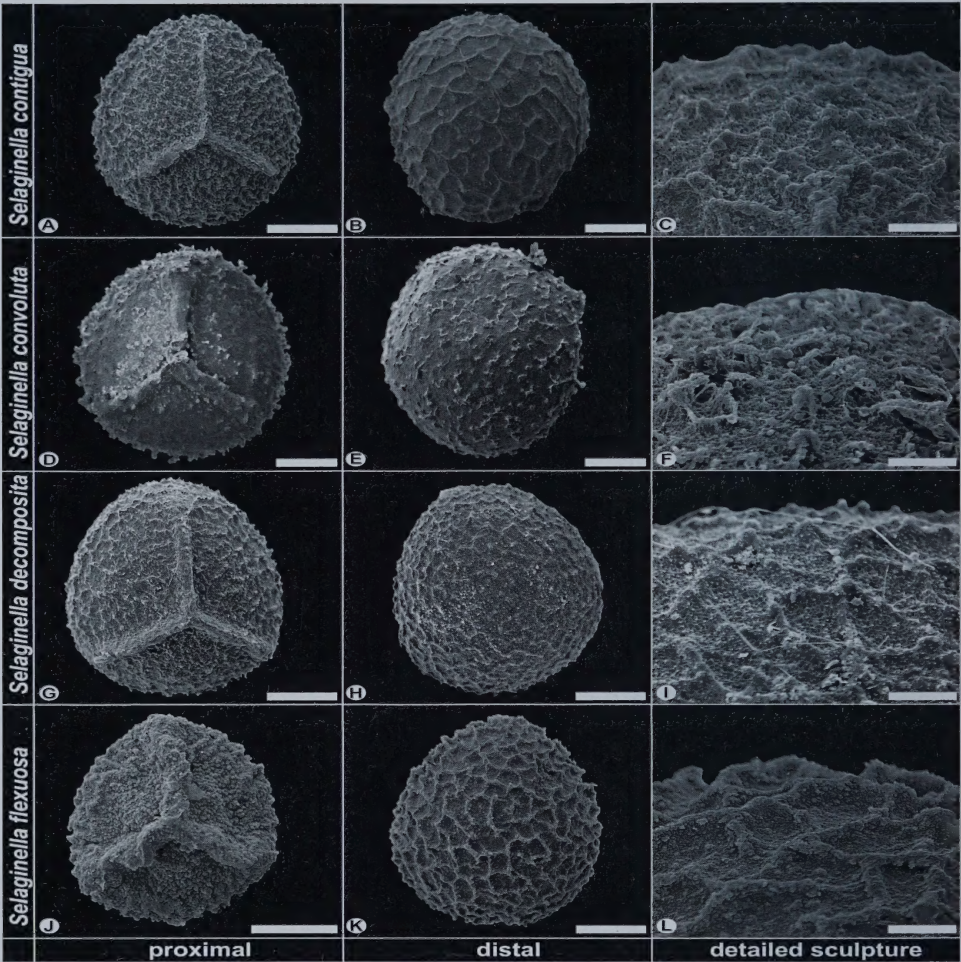


FIG. 1. Megaspores of *S. contigua* (A–C), *S. convoluta* (D–F), *S. decomposita* (G–I), and *S. flexuosa* (J–L). Scale bar proximal and distal faces 100µm, scale bar detailed sculptures 40µm.

Megaspores globose, white, 300–370 µm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture simple, approximate wide as high, 210 µm in diameter; laesurae extending to 7/8 of the distance to the equator, pronounced, straight, coarse, not wing-like, increasing towards the pole, more or less complex mass at the pole, scabrate, not foveolate. Reticulum (proximal faces) with 7–8 muri/100 µm, irregular, open; muri 5–10 µm high, 5–10 µm wide, porous, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) clavate, gemmate, baculate, rugulate, striate, not foveolate; microsculpture (proximal faces) scabrate, micro-rugulate, micro-perforate. Reticulum (distal faces) with 23 muri/100 µm, irregular, mostly open; muri 10–15 µm high, 5–10 µm wide, porous, mostly acute; the sides of most muri meeting the underlying lumina at

a nearly right angle; macrosculpture (distal faces) clavate, verrucate, rugulate, not foveolate; microsculpture (distal faces) scabrate, micro-rugulate, micro-perforate.

Distribution and habitat.—*Selaginella contigua* is endemic to Brazil and recorded for the states: São Paulo, Rio de Janeiro, Minas Gerais, Paraná, and Santa Catarina. It grows in forests and near forest edges in humus layers on mesic clay and on shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Nova São Paulo-Santos (Via Anchieta) Alto da Serra, 14 Oct 1961, *E. Pereira et al.* 5966 (R)*. Santos, 10 Feb 1875, *C.W.H. Mosén* 3556 (K). Bananal, 26 Sep 1978, *A.T. Silva* 120 (SP)*. Caraguatatuba, 15 Jul 1953, *F.C. Hoehne* 4112a (SP*, SPF, CESJ). Santa Izabel, 29 Sep 1936, *J.G. Kuhlmann s.n.* (SP*, SPF). Santo André, Alto da Serra, Estação Biológica de Paranapiacaba, 26 Oct 1954, *O. Handro* 413 (SP*, SPF). São José do Barreiro, Fazenda São Miguel, Apr 1894, *A. Löfgren and G. Edwall s.n.* (SP)*. São Vicente, Jun 1941, *F.C. Hoehne* 669 (SPF). Rio de Janeiro: Itatiaia, 30 Nov 1985, *O. Cesar* 647 and *A. Feddersen Jr.* 058 (HRCB*). Itatiaia, Lago Azul, 1 Apr 1959, *B. Flaster* 10 (R)*. Parque Estadual Carlos Botelho Núcleo de Sete Barras, Mun. Sete Barras [24°12'S, 47°55'W], 27 Sep 2002, *V.A. de O. Dittrich and T. B. Breier* 1204 (HRCB)*. Itatiaia, Maromba, 25 Jun 1930, *A.C. Brade* 10203 (R)*. s.l., 18 Jul 1873, *C.W.H. Mosén* 53 (K). s.l., Dec 1878, *A.F.M. Glaziou* 9314 (K). Parque Nacional do Itatiaia, 2 Jul 1966, *A. Lourteig* 1833, 1834 (P). s.l., 20 Mar 1907, *A.F.M. Glaziou s.n.* (P). Serra da Bocaina, 1879, *A.F.M. Glaziou and C.A.W. Schwacke s.n.* (P). s.l., 1901, *H. Christ s.n.* (P). Serra do Itatiaia, 17 Jul 1902, *P. Dusén* 708 (P). Tijuca, 27 Feb 1878, *A.F.M. Glaziou* 9312 (P). Parque Nacional do Itatiaia, Trilha para os Três Picos [22°26'5"S, 44°36'12"W], 2 Jul 2008, *E.R. Damasceno* 178 (RB). Mun. de Resende, Parque Nacional do Itatiaia, Rio Campo Belo, próximo da Cachoeira Véu de Noiva, 1 May 1985, *G. Martinelli et al.* 10762 (RB). Minas Gerais: Nova Lima, Mata da Mutuca, 15 Mar 2003, *F.A. Carvalho et al.* 108 (BHCB, HRCB*). Espírito Santo: Cariacica Localidade de Alegre, trilha do Pauoco [20°16'4"S, 40°31'30"W], 22 Jul 2008, *P.H. Labiak* 4899 (RB). Parana: Parque Estadual das Lauráceas, Mun. Adrianópolis, 12 Jan 2000, *V.A. de O. Dittrich, et al.* 730 (HRCB)*.

Selaginella convoluta (Arn.) Spring, Fl. Bras. 1(2):131. 1840. *Lycopodium convolutum* Arn., Mem. Wern. Nat. Hist. Soc. 5:199. 1824. Fig. 1 D–F.

Megaspores globose, creamy white, 340–380 µm in diameter, heteropolar, without curvaturae perfectae; trilete aperture simple, wider than high, 230 µm in diameter; laesurae extending to 2/3 of the distance to the equator, pronounced, smooth, not wing-like, straight, increasing towards the pole, without complex mass, scabrate, not foveolate. Reticulum (proximal faces) absent, without additional ornamentation; macrosculpture (proximal faces) baculate, rugulate, verrucate, not foveolate; microsculpture (proximal faces) scabrate, micro-perforate. Reticulum (distal faces) absent; macrosculpture

(distal faces) baculate, clavate, rugulate, verrucate, without additional ornamentation, not foveolate; microsculpture (distal faces) scabrate, micro-perforate.

Distribution and habitat.—*Selaginella convoluta* is distributed in Mexico, Cuba, Hispaniola, Guatemala, Venezuela, Guiana, French Guiana, Colombia, Bolivia, Argentina, and recorded in Brazil for the states: Piauí, Ceará, Paraíba, Pernambuco, Bahia, Goiás, Minas Gerais, Espírito Santo, Rio de Janeiro, and São Paulo. It grows in open areas, on rocks, and in sandy soils.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Salto Grande, Parapanema, Sep 1906, *G. Edwall s.n.* (SP)*. Monte Alto, Serra Tabarana, 3 Jun 1995, *M.R. Pietrobon da Silva and F.R. Nonato 1780* (MBM). Rio de Janeiro: Nova Friburgo, 1 Sep 1947, *J.E. Leite 4209* (F)*. s.l., Jul 1887, *E. Ule 233* (P). Minas Gerais: Santo Hipólito: Estrada para Monjolos a 4.3 km do mata-burro e porteira na saída da cidade, 25 Mar 1997, *J.R. Pirani et al. 3819* (F, SPF)*. Rio Novo, Faz. de Santana, 13 May 1972, *L. Krieger 329* (R)*. Rio Novo, Faz. De Santana, 3 Dec 1984, *L. Krieger and R.F. Novelino-Camargo 375* (R)*. Espírito Santo: Entre Colatina e Patrimônio 15, 4 Dec 1971, *A.P. Duarte 13992* (RB). [20°52'S, 40°46'W], 18 Jan 1993, *J.A. Kallunki and J.R. Pirani 331* (SPF). Piauí: Mun. São Raimundo Nonato, S. João Vermelho, 20 Jan 1984, *L. Empeiane 200* (P). Pernambuco: Sertania, Fazenda Peter Penic, 20 Jan 1976, *J.L. Waechter 447* (ICN). [08°26'58"S, 39°25'35"W], 22 Jan 2009, *J.R. Maciel et al. 709*, (UPCB)*. Tapera, 14 Sep 1933, *D.B. Pickel 58* (P). Imenara, 3 Apr 1979, *G.T. Rizzini 1119* (RB). Unaí, Área de Cerrado e Mata Ciliar do Rio São Miguel, na região da barra do Córrego Bebedouro, 11 Dec 2001, *A. Salino and F.A. Carvalho 7869* (RB). [18°17'S, 44°11'W], 4 Apr 1996, *J.R. Pirani, et al. 3737* (SPF). [17°56'S, 44°09'W], 11 Oct 1988, *R.M. Harley 25614* (SPF). [15°23'00"S, 42°45'3235"W], *P.H. Labiak 599* (UPCB)*. Bahia, [10°41'S, 39°48'W], 19 Feb 1974, *R.M. Harley 16181* (P). [12°19'4"S, 42°54'24"], 15 Nov 2012, *E.L.M. Assis et al. 1084* (RB). [10°38'S, 39°42'W], 1 Dec 1998, *M.M. Arbo et al. 5472* (SPF). [13°38'S, 41°50'W], 12 Dec 1988, *R.M. Harley 27130* (SPF). Mun. Uauá, 1.7 km ESE of Uauá towards Bendengó and Canudos, Rio Vaza Barris, 6 Jan 1991, *N.P. Taylor et al. 1364* (HRCB)*.

Selaginella decomposita Spring in Mart., Fl. Bras. 1(2):123. 1840. Fig. 1 G–I.

Megaspores globose, white, 300–320 μm in diameter, heteropolar, without curvaturae perfectae; trilete aperture simple, higher than wide, 240 μm in diameter; laesurae extending to 7/8 of the distance to the equator, pronounced, straight, smooth, not wing-like, the whole length the same height, without complex mass, verrucate, scabrate, not foveolate. Reticulum (proximal faces) with 56 muri/100 μm , irregular, mostly open; muri 0–5 μm high, 0–5 μm wide, porous, mostly acute; the sides of most muri sloping gradually to meet the underlying lumina; macrosculpture (proximal faces) reticulate, rugulate-cristate, rugulate; microsculpture (proximal faces) micro-verrucate, scabrate, micro-rugulate, micro-perforate. Reticulum (distal faces) with 6–11 muri/100 μm , irregular, mostly open; muri 5–10 μm high, 0–5 μm wide, porous, mostly

acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) reticulate, rugulate, rugulate-cristate, not foveolate; microsculpture (distal faces) micro-verrucate, scabrate, micro-rugulate, micro-perforat.

Distribution and habitat.—*Selaginella decomposita* is endemic to Brazil and recorded for the states: Pernambuco, Mato Grosso, Minas Gerais, Bahia, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina. It grows in forests and near forest edges, in humus layers on mesic clay and on shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Apiaí, s.d., *J. Puiggari* 2385 (BM, RB). Bananal, Nova Suíça, 26 Sep 1978, *Tosta Silva* 130 (SP)*. Itaberá, 12 Jan 1983, *J.R. Pirani et al.* 385 (SP)*. Salesópolis, Estação Biológica de Boracéia, 14 Feb 1999, *P.H. Labiak* 981 (SP)*. Santo André, Alto da Serra, Estação Biológica de Paranapiacaba, 16 Jun 1998, *R.Y. Hirai et al.* 92 (SP)*. São Luiz do Paraitinga, Parque Estadual da Serra do Mar, Núcleo de Santa Virgínia, 19 Nov 1998, *C. Kameyama et al.* 127 (SP, SPF)*. [23°49'32,8-38,0"S, 46°56'22,3-30,7"W], 28 Sep 2010, *J.A. Lombardi et al.* 7853 (HRCB)*. Rio de Janeiro: Therezopolis, Cascata feroz, 2 Oct 1929 *A.C. Brade s.n* (R)*. Serra do Itatiaia Chuch. Maromba, 25 Jun 1930, *A.C. Brade* 10190 (R)*. St. Antonio de Imbré, 1 Apr 1932, *A.C. Brade* 11663, 11798, 10163 (F)*. Terezópolis, 18 Sep 1948, *D.B. Lutz s.n* (R)*. Parque Nacional do Itatiaia, veu da Novia, 2 Jul 1966, *A. Lourteig* 1835, 1836 (R)*. Minas Gerais: Rio Preto, Serra Negra, Serra da Caveira, Faz. da Tiririca, 21 Jan 2006, *F.S. Souza et al.* 121 (SP*, CESJ, BHCB, UPGB*). Rio Preto, Serra Negra Ninho da Égua, 20 May 2006, *F.S. Souza et al.* 209 (MBM). Espírito Santo: [20°16'4"S, 40°31'30"W], 22 Jul 2008, *P.H. Labiak et al.* 4888 (RB). Castelo Forno Grande, 18 May 1949, *A.C. Brade* 19855 (RB). Cachoeiro de Itapemirim Vargem Alta. Córrego dOuro, 23 May 1949, *A.C. Brade* 19988 (RB). Paraná: Jaguaríava, Parque Estadual do Cerrado, 3 Sep 1994, *P.H. Labiak* 172 (UPGB)*. Bahia: [15°23'30"S, 39°33'55"W], 3 Mar 2006, *F.B. Matos* 1069 (UPGB)*.

Selaginella flexuosa Spring, *Flora* 21:197. 1838. *Lycopodioides flexuosa* (Spring) Kuntze, *Rev. Gen. Pl.* 1:826. 1891. Fig. 1 J–L.

Megaspores globose to subtriangular, white, 200–280 µm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture simple, higher than wide, 150 µm in diameter; *laesurae* extending to 7/8 of the distance to the equator, indistinct, coarse, slightly sinuous, not wing-like, increasing towards the pole, without complex mass, scabrate, not foveolate; sculpture in the center, close to the *laesurae* differs to the sculpture along the edge. Reticulum (proximal faces) with 7–10 muri/100 µm, irregular, mostly closed; muri 10–15 µm high, 0–5 µm wide, mostly blunt; the sides of most muri sloping gradually to meet the underlying lumina; macrosculpture (proximal faces) gemmate, rugulate, rugulate-cristate, not foveolate; microsculpture (proximal faces) micro-gemmate, scabrate, with thin and fine fissure. Reticulum (distal faces) with 5–6 muri/100 µm, irregular, more or less closed; muri 5–10 µm high,

0–5 μm wide, coarse, mostly acute; the sides of most muri sloping gradually to meet the underlying lumina; macrosculpture (distal faces) gemmate, rugulate-cristate, without additional ornamentation, not foveolate; microsculpture (distal faces) micro-gemmate, scabrate.

Distribution and habitat.—*Selaginella flexuosa* is endemic to Brazil and recorded for the states: Pernambuco, Mato Grosso, Minas Gerais, Bahia, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina. It grows in forests and near forest edges, in open grasslands shadowed by vegetation. It grows in humus layers on clay and on shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Serra do Paranapiacaba, 2 Aug 1941, A. Lutz 1876 (R)*. Apiaí, s.d., J. Puiggari s.n. (BM, RB). Iguape, Estação Ecológica Juréia-Itatins, Serra da Juréia, 19 Sep 1990, I. Cordeiro et al. 714 (SP)*. Salesópolis, Estação Biológica de Boracéia, 5 Mar 1962, O.P. Travassos 377, 404 (RB). Santo André, Alto da Serra, Estação Biológica de Paranapiacaba, 16 Jun 1998, R.Y. Hirai et al. 94, 96 (SP)*. [23°30'31.1"S, 45°11'58.8"W], 22 Jun 2006, P. Fiaschi and A. Reydon 3033 (SPF). Parque Estadual Intervales, Base de Saibadela, Mun. Sete Barras, 14 Dec 2000, V.A. de O. Dittrich and C. von Allmen 825 (HRCB)*. [24°12'–24°15'S, 48°03'–48°06'W], 13 Nov 2001, V.A. de O. Dittrich 1008 (HRCB)*. Rio de Janeiro: Sena da Banaca, Jul 1929, A.C. Brade s.n. (R)*. Serra do Carioca, Jul 1929, A.C. Brade s.n. (R)*. Serra do Carioca, 19 Nov 1928, L.B. Smith 1278 (F)*. Mun. Petrópolis, Estrada fazenda Inglesa, Pati do Alferes, 22 Apr 1980, T. Plowman and G. Martinelli 10143 (F)*. Minas Gerais: Serra do Cipó, 6 Mar 1958, E.P. Heringer and A. Castellanos 22216 (R)*. [18°55'48"S, 43°40'17"W], 20 Jan 2007, J.R. Pirani et al. 5605 (SPF). Rio Preto, RPPN Mato Limpo, Gruta dos Macacos, 6 Oct 2007, F.R.G. Salimena and P.H. Nobre 2732 (MBM). Paraná: Ypiranga, 14 Feb 1904, P. Dusén 3720 (R)*. Rio Ipiranga, Mun. Morretes, 26 Jun 1966, G. Hatschbach 14474 (F)*. Morretes, Parque Estadual Pico do Marumbi, 06 Dec 1998, V.A. de O. Dittrich 551 (ICN). [25°21'55"S, 48°52'31"W], 8 Sep 2005, P.H. Labiak and M.L.B. Paciência 3579 (UPCB)*. Santa Catharina: Blumenau, 1884, C.A.W. Schwacke 6 (R)*. Bahia: [14°46'13"S, 39°12'10"W], 13 Jul 2005, F.B. Matos et al. 678 (MBM).

Selaginella kraussiana (Kunze) A. Braun, Index Sem. Hort. Bot. Berol. p. 22. 1860. *Lycopodium kraussianum* Kunze, Linnaea 18:114. 1844. Fig. 2 A–C.

Megaspores globose, white, 600–650 μm in diameter, heteropolar, without curvaturae perfectae; trilete aperture simple, higher than wide, 220 μm in diameter; laesurae extending to 1/2 of the distance to the equator, indistinct, sinuous, smooth, not wing-like, increasing towards the pole, without complex mass, scabrate, not foveolate. Reticulum (proximal faces) with 12 muri/100 μm , irregular, mostly open; muri 5–11 μm high, 0–5 μm wide, porous, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) clavate, baculate, gemmate, verrucate, striate, rugulate, not foveolate; microsculpture (proximal faces) micro-clavate,

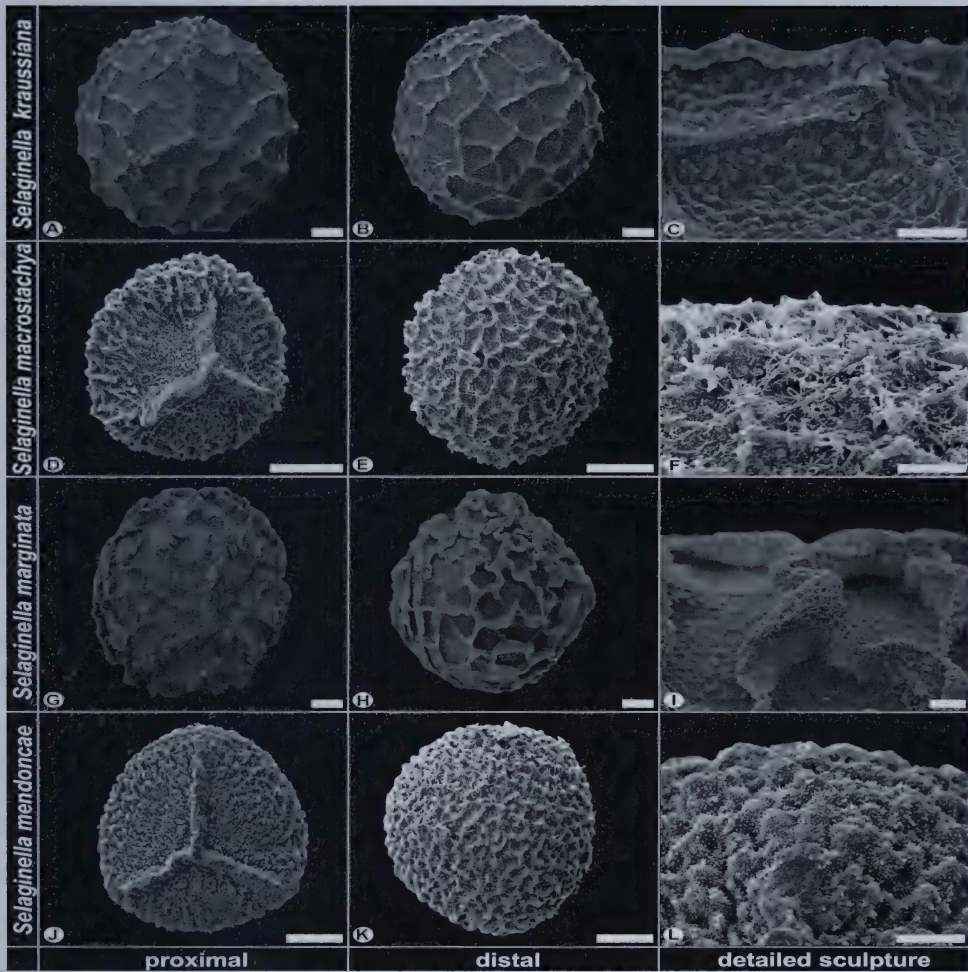


FIG. 2. Megaspores of *S. kraussiana* (A–C), *S. macrostachya* (D–F), *S. marginata* (G–I), and *S. mendoncae* (J–L). Scale bar proximal and distal faces 100 μ m, scale bar detailed sculptures 40 μ m.

micro-gemmate. Reticulum (distal faces) with 1–2 muri/100 μ m, irregular, mostly open; muri 5–10 μ m high, 0–5 μ m wide, porous, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) gemmate, verrucate, rugulate, not foveolate; microsculpture (distal faces) micro-clavate, micro-gemmate.

Distribution and habitat.—*Selaginella kraussiana* is native to Africa and Macronesia. It is recorded in Brazil for the states: Minas Gerais, Rio de Janeiro, São Paulo, Santa Catarina, and Rio Grande do Sul. It grows in forests and near forest edges in humus layers on mesic clay close to rivers and in wet areas.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Cantareira, 24 Apr 1933, W. Hoehne 222 (CESJ, SPF). Campos do Jordão, 27 Oct 1992, C. Curra

et al. 9066 (MBM). Santo André, Paranapiacaba, 3 Sep 1976, *P.H. Davis et al.* 60507 (UEC). Rio Claro, culture in the greenhouse, 4 Feb 2013, *D.S. Bauer* 26 (HRCB)*. Rio Grande do Sul: Floricultura, pequeno barranco no mato Grama do, 18 Sep 1971, *J.C. Lindeman et al. s.n.* (ICN). Mun. St. Cruz, 1925, *L. Jürgens* 35 (ICN).

Selaginella macrostachya (Spring) Spring, Bull. Acad. Roy. Sci. Brux. 10:144. 1843. *Selaginella flexuosa* ssp. *macrostachya* Spring in Mart., Fl. Bras. 1(2):123. 1840. Fig. 2 D–F.

Megaspores globose, white, 270–310 μm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture simple, approximate wide as high, 160 μm in diameter; laesurae extending to 7/8 of the distance to the equator, pronounced, slightly sinuous, coarse, not wing-like, the whole length the same height, without complex mass, scabrate, perforate, not foveolate; sculpture in the center, close to the laesurae differs to the sculpture along the edge. Reticulum (proximal faces) with 5–6 muri/100 μm , regular, more or less closed; muri 5–10 μm high, 0–5 μm wide, coarse, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) clavate, rugulate, rugulate-cristate, perforate, not foveolate; with additional ornamentation; microsculpture (proximal faces) scabrate, micro-perforate. Reticulum (distal faces) with 4 muri/100 μm , regular, closed; muri 10–15 μm high, 0–5 μm wide, coarse, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) rugulate, rugulate-cristate, perforate, with additional ornamentation, not foveolate; microsculpture (distal faces) scabrate, micro-perforate.

Distribution and habitat.—*Selaginella macrostachya* is endemic to Brazil and recorded for the states: Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, and Santa Catarina. It grows in forests and near forest edges in humus layers on mesic clay.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Santos, 30 Mar 1875, *C.W.H. Mosén* 3758 (R)*. Iguape, Serra do Itatins, Mar 1924, *A.C. Brade* 8627 (RB). Salesópolis, Exp. de Boraceia, Picada da Cachoeira do Pilão, 05 Mar 1962, *O.P. Travassos* 378, 400, 402 (RB). Cananéia, Ilha do Cardoso, 12 Jan 1977, *A. Tosta Silva* 29 (SP)*. Iguape, Estação Ecológica de Juréia, 15 Aug 1990, *J. Prado et al.* 317 (SP)*. Santo André, Alto da Serra, Estação Biológica de Paranapiacaba, 16 Jun 1998, *R.Y. Hirai et al.* 91, 93, 95 (SP)*. Paranapiacaba, Estação Biológica, 28 Sep 1955, *O. Handro* 527 (SPF). Rio de Janeiro: Alto Macahé, 22 Jan 1874, *A.F.M. Glaziou* 7284 (F)*. Teresópolis, Serra dos Órgãos, 2 Feb 1983, *J.E. Simonis and G. Martinelli* 32 (RB). Nova Friburgo, Morro da Caledônia, 8 Jun 1977, *G. Martinelli* 2535 (RB). Teresópolis, Serra dos Órgãos, Oct 1958, *R. Schnell* 8339 (RB). [22°27'31"S, 43°00'18"W], 23 Feb 2005, *R. Engelmann* 230 (SPF). [22°29'38.2" S, 43°00'04.9"W], 15 Apr 2011, *J.A. Lombardi et al.* 8475 (HCRB)*. Minas Gerais: s.l., s.d., *Lindberg s.n.* (K). Lima Duarte, Parque Estadual do Ibitipoca, Subindo da Cachoeira da Pedra Quadrada pela trilha

inferior esquerda, 15 May 1993, *R.F. Novelino 1062* (RB). Espírito Santo: [18°47'13"S, 40°26'44"W], 15 Jan 2009, *P.H. Labiak et al. 5131* (RB). [20°14'32"S, 40°31'20"W], 17 Jan 2009, *P.H. Labiak et al. 5156* (RB).

Selaginella marginata (Kunth) Spring, *Flora* 21:194. 1838. *Lycopodium marginatum* Kunth Sp. pl. 5:41. 1810. Fig. 2 G–I.

Megaspores globose, white, 590–650 μ m in diameter, subisopolar, without *curvaturae perfectae*; trilete aperture simple, approximate wide as high, 380 μ m in diameter; *laesurae* extending to 3/4 of the distance to the equator, pronounced, straight, coarse, not wing-like, the whole length the same height, without complex mass, scabrate, perforate, not foveolate. Reticulum (proximal faces) with 1–2 muri/100 μ m, irregular, mostly open; muri 15–30 μ m high, 15–20 μ m wide, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) verrucate, rugulate, striate, without additional ornamentation, not foveolate; microsculpture (proximal faces) scabrate, micro-clavate, micro-perforate. Reticulum (distal faces) with 1 muri/100 μ m, regular, closed; muri 15–20 μ m high, 5–20 μ m wide, coarse, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) rugulate, without additional ornamentation, not foveolate; microsculpture (distal faces) scabrate, micro-clavate, micro-perforate.

Distribution and habitat.—*Selaginella marginata* is distributed in Mexico, Venezuela, Bolivia, Uruguay, Argentina, Paraguay and recorded in Brazil for the states: Maranhão, Piauí, Bahia, Mato Grosso, Goiás, Minas Gerais, Mato Grosso do Sul, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul. It grows in forests and near forest edges in humus layers on mesic clay and shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Barreiro, Serra da Bocaina, Pinheirinho, Mar 1902, *Segadas-Vianna 3006* (R)*. Campos da Bocaina, 25 Nov 1950, *A.C. Brade 20549* (RB). Campos do Jordão, 28 Jun 1998, *P.H. Labiak 671, 672* (SP)*. Lorena, Salto Santa Thereza, Fazenda Águas de Santa Rosa, Apr 1924, *H. Luederwaldt s.n.* (BM, SP*, SPF). [23°18'42"S, 45°07'11.4"W], 3 Nov 2001, *A. Salino et al. 7855* (HRCB)*. [22°43'S, 45°27'W], 2 Apr 2002, *V.A.O. Dittrich 1112* (HRCB)*. Rio de Janeiro: Teresópolis Serra dos Órgãos, 21 Apr 1941, *A.C. Brade 16811* (RB). Minas Gerais: Santana do Riacho, Serra do Cipó acesso pela Faz. Inhame, Propriedade do Sr. Geraldo Alves Ferreira a 2,2 Km da ponte do Rio das Pedras, 28 Feb 2009, *C.N. Fraga 2438* (RB). Ituiutaba, São Lourenço, 1 Apr 1957, *A. Macedo 4927* (SPF). [17°56'S, 44°09'W], 11 Oct 1988, *R.M. Harley et al. 24837* (SPF). Paraná: s.l., 13 Jun 1914, *P.K.H. Dusén 5288* (F)*. Paraná, Serra do Mar, Ypirana, 16 Jan 1914, *P.K.H. Dusén 14435* (F)*. Jaguariahyra, Fazenda Samambarya, 12 May 1914, *P.K.H. Dusén 15020* (F)*. São Mateus do Sul, Margem do Rio Iguaçu, 18 Apr 2005, *R. Wasum 2609* (ICN). Rio Capivara, 5 Jul 1997, *O.S. Ribas and L.B.S. Pereira 1895* (HRCB)*. Santa Catarina: Campo dos Padres, 16 Dec 1948, *R. Reitz 2397* (RB). Rio Grande do

Sul: Bom Jesus, Serra da Rocinha, 4 Nov 1911, *R. Bueno s.n.* (ICN). Goiás: [51°04'W, 18°36'S], 13 Nov 1994, *M.R. da Silva 1494* (SPF).

Selaginella mendoncae Hieron. in Engl. & Prantl, Nat. Pflanzenfam. 1(4):693. 1901. Fig. 2 J–L.

Megaspores globose, white, 290–310 μm in diameter, heteropolar, without curvaturae perfectae; trilete aperture approximate wide as high, with thickened ends, 215 μm in diameter; laesurae extending to 7/8 of the distance to the equator, indistinct, smooth, straight, the whole length the same height, without complex mass, verrucate, scabrate, not foveolate. Reticulum (proximal faces) absent; macrosculpture (proximal faces) clavate, gemmate, verrucate, rugulate-cristate, perforate, without additional ornamentation, not foveolate; microsculpture (proximal faces) micro-echinate, scabrate, micro-rugulate, micro-perforate. Reticulum (distal faces) with 5–6 muri/100 μm , regular, closed; muri 0–5 μm high, 5–10 μm wide, coarse, mostly blunt; the sides of most muri sloping gradually to meet the underlying lumina; macrosculpture (distal faces) clavate, rugulate, rugulate-cristate, without additional ornamentation, not foveolate; microsculpture (distal faces) scabrate, micro-perforate.

Distribution and habitat.—*Selaginella mendoncae* is endemic to Brazil and recorded in Rio de Janeiro and São Paulo. It grows in forests and near forest edges in humus layers on mesic clay and shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Juquitiba, Fazenda Iterei, 2 Nov 1994, *J. Prado et al. 522* (SP)*. Miracatu, Fazenda Iterei, Serra de Paranapiacaba, 19 Apr 1994, *J.R. Pirani and Garcia 3103* (SP, SPF)*. Rio de Janeiro: Serra dos Órgãos, 24 Mar 1880, *A.F.M. Glaziou 12294* (F)*. Parque Nacional da Serra dos Órgãos, Santo Aleixo, 3 May 2011, *R.A. Engelmann and J. Wesenberg 1769, 1795* (RB). Teresópolis Serra dos Órgãos, s.d., *C.A.W. Schwacke 4361* (RB). Guapimirim Granja Monte Olivete, trilha das Andorinhas, margem do afluente do Rio Bananal. Guapimirim, Cachoeiras de Macacu, 7 Jun 1995, *J.M.A. Braga 2467* (RB). Cachoeiras de Macacu Nova Friburgo, 5 May 1993, *C.M. Mynssen 15* (RB).

Selaginella microphylla (Kunth) Spring, Bull. Acad. Roy. Sci. Brux. 10:234. 1843. *Lycopodium microphyllum* Kunth in Humb., Bonpl. & Kunth, Nov. gen. sp. 1:39. 1816. Fig. 3 A–C.

Megaspores globose to subtriangular, white, 180–200 μm in diameter, heteropolar, indistinct, curvaturae perfectae; trilete aperture simple, approximate wide as high, 110 μm in diameter; laesurae extending to 3/4 of the distance to the equator, pronounced, straight, coarse, not wing-like, increasing towards the pole, without complex mass, scabrate, not foveolate; sculpture in the center, close to the laesurae differs to the sculpture along the edge. Reticulum (proximal faces) with 2–12 muri/100 μm , irregular, closed; muri 5–10 μm high, 0–5 μm wide, mostly acute; the sides of most muri sloping gradually to meet the underlying lumina; macrosculpture (proximal faces) verrucate, striate, reticulate,

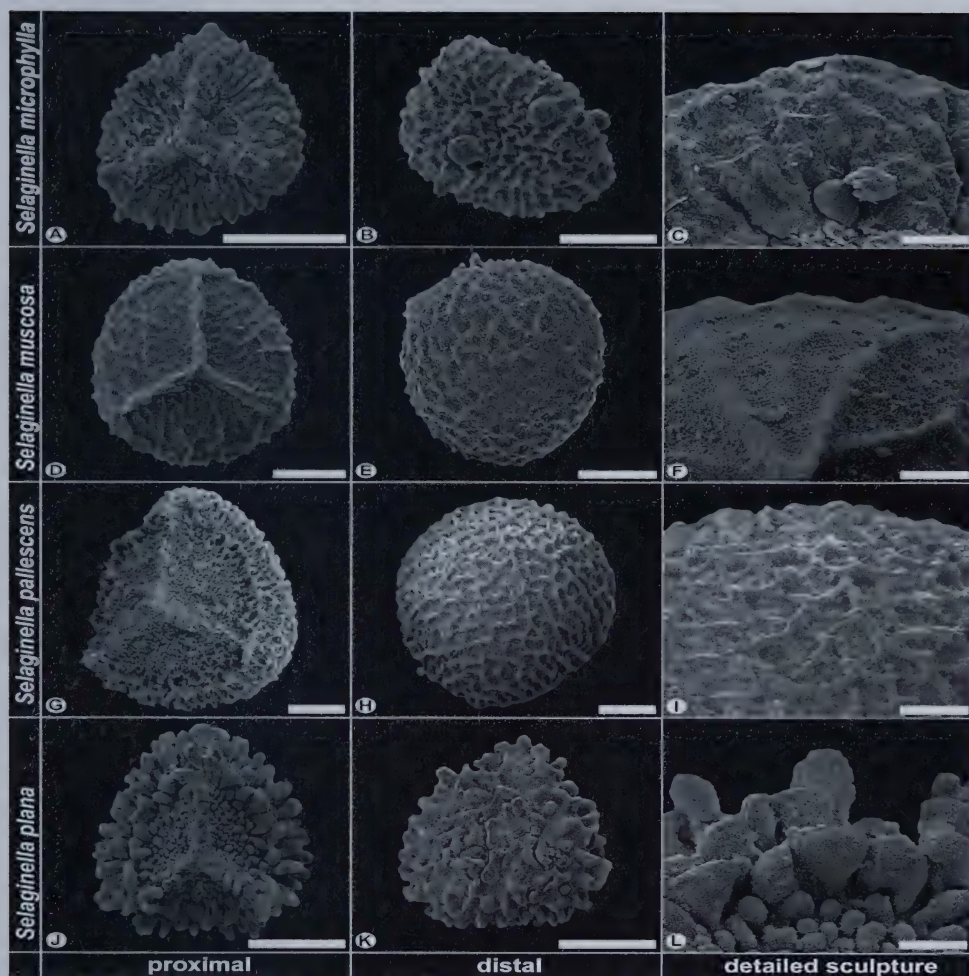


FIG. 3. Megaspores of *S. microphylla* (A–C), *S. muscosa* (D–F), *S. pallescens* (G–I), and *S. plana* (J–L). Scale bar proximal and distal faces 100 μ m, scale bar detailed sculptures 40 μ m.

rugulate, fossulate, not foveolate; microsculpture (proximal faces) micro-verrucate, micro-gemmate, micro-rugulate, scabrate, micro-perforate. Reticulum (distal faces) with 2–15 muri/100 μ m, irregular, closed; muri 5–10 μ m high, 0–10 μ m wide, coarse, mostly acute; the sides of most muri sloping gradually to meet the underlying lumina; macrosculpture (distal faces), rugulate, perforate, not foveolate; microsculpture (distal faces) micro-clavate, micro-verrucate, micro-gemmate, micro-rugulate, scabrate, micro-perforate.

Distribution and habitat.—*Selaginella microphylla* is distributed in Costa Rica, Panama, Colombia, Venezuela, Ecuador, Paraguay, Uruguay, Argentina, Bolivia, Peru, and recorded in Brazil for the states: Minas Gerais, Rio de Janeiro, São Paulo, Santa Catarina, and Rio Grande do Sul. It grows on sandy humus soil and on shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Campos de Jordão, 19 Apr 1905, *T. Campos Torto* 3113 (RB). Itirapina, Morro Pelado, 6 Oct 1976, *T. Silva* 8 (SP)*. Amparo, Monte Alegre, 26 Aug 1943, *J.G. Kuhlmann* 466(SP, SPF)*. Serra de Botucatu, Fazenda Nova América, 31 Jul 1935, *F.C. Hoehne and A. Gehrt s.n.* (BM, HB, SP*, SPF). [48°47'W, 22°17'S], 22 May 1993, *M.R. Silva and R.M.C. Andrade* 952 (SPF). [22°50'S, 48°30'W], 06 May 2011, *J. Prado et al.* 2144 (HRCB)*. [20°12'46.12"S, 47°44'13.48"W], 12 Feb 2013, *D.S. Bauer* 22 (HRCB)*. Rio de Janeiro: s.l., s.d., *A.F.M. Glaziou* 7667 (B). Minas Gerais: Ouro Preto, 1893, *C.A.W. Schwacke* 9169 (RB). Santa Catarina: Rio Pirahy Mirim, 1897, *C.A.W. Schwacke* 13227 (RB). Itajaí Praça Serafim L. Alves, 2 Jan 1948, *Reitz* C2036 (RB). Arar Serra da Pedra, 28 Dec 1949, *Reitz* C349 (RB). Rio Grande do Sul: Forromeco, s.d., *A. Kunert s.n.* (P). Farroupilha, ad rupes iuxta rivum, 7 Apr 1953, *A. Sehnem* 6444 (B). Mun. S. Cruz, Faz. Leitãs, 1906, *C. Jürgens s.n.* (B). Santa Catharina, São Bento, 24 Nov 1886, *J.H.R. Schenck* 1302 (B). Sta Anna, 26/28 May 1907, *W.G.F. Herter* 3056 (B). Mato Grosso do Sul: Chapada dos Guimarães, Na Cachoeirinha, ca. de 60 km de Cuiabá, 30 Apr 1981, *G. Guarim Neto et al.* 364 (UFMT, HRCB*).

Selaginella muscosa Spring in Mart. Fl. Bras. 1(2):120. 1840. *Lycopodioides muscosa* (Spring) Kuntze Rev. Gen. Pl. 1:827. 1891. Fig. 3 D–F.

Megaspores globose, creamy white, 250–290 μm in diameter, heteropolar, without curvaturae perfectae; trilete aperture simple, higher than wide, 220 μm in diameter; laesurae extending to 7/8 of the distance to the equator, pronounced, straight, coarse, not wing-like, descending towards the pole, without complex mass, scabrate, not foveolate; sculpture in the center, close to the laesurae differs to the sculpture along the edge. Reticulum (proximal faces) with 4–6 muri/100 μm , irregular, open; muri 0–15 μm high, 5–10 μm wide, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) echinate, gemmate, reticulate, rugulate, not foveolate; microsculpture (proximal faces) micro-clavate, micro-verrucate, scabrate, micro-perforate. Reticulum (distal faces) with 4–6 muri/100 μm , regular, open; muri 5–15 μm high, 5–20 μm wide, porous, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) rugulate, without additional ornamentation, not foveolate; microsculpture (distal faces) micro-clavate, micro-gemmate, scabrate, micro-perforate.

Distribution and habitat.—*Selaginella muscosa* is distributed in Venezuela, Trinidad, Tobago, Colombia, Peru, Paraguay, Argentina, Uruguay, Ecuador, British Guyana, and is recorded in Brazil for the states: Ceará, Amazonas, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul. It grows in forests and near forest edges in humus layers on mesic clay close to rivers and wet areas. *Selaginella muscosa* grows in the same habitat as *Selaginella kraussiana* and they are often observed together.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Iporanga, Ribeira do Iguape, Oct 1896, *A. Löfgren and G. Edwall s.n.* (R)*. Apiaí, s.d., *J. Puiggari 2383, 2384* (R)*. Sete Barras, Sete Barras-Eldorado, 27 Oct 1997, *R.Y. Hirai and O.S. Ribas 73* (SP)*. São Luiz do Paraitinga, Fazenda Rio das Flores, 11 Jun 1982, *Yano 4309* (SP)*. Monte Alegre do Sul, 27 Jul 1949, *J.G. Kuhlmann 1898* (SP)*. Itararé, Ibiti, Fazenda Ponte Alta, 29 Jul 1946, *J.G. Kuhlmann 1394* (SP*, SPF). Guarujá, Forte dos Andradas, 17 Sep 1994, *Yano and Mello 23114* (SP)*. Silveiras Estrada do Parque Nacional da Serra da Bocaina para Campos Novos, 7 Jan 2008, *P.H. Labiak 4335* (RB). [45°05'W, 23°26'S], 18 Nov 1993, *M.R. da Silva 1217* (SPF). [46°37'W, 24°06'S], 2 Aug 1997, *F.P.F. Athayde 195* (SPF). Teodoro Sampaio, Morro do Diabo, 16 Jan 1995, *M.R. Pietrobon-Silva 1555* (SPF). [24°12'-24°15'S, 48°03'-48°06'W], 13 Nov 2001, *V.A. de O. Dittrich 1000* (HRCB)*. Município de Ubatuba, Parque Estadual da Serra do Mar, Núcleo Picinguaba, 10 Nov 1993, *A. Salino 1993* (HRCB)*. Ubatuba, Picinguaba, 4 Nov 1988, *A. Furlan et al. 550* (HRCB*, SJRP, SP*). Rio de Janeiro: Itatiaia Margem do Rio Campo Belo, entre o Lago Azul e o Rio Taquaral, 28 Apr 2005, *L. Sylvestre 1825* (RB). Paraná: Mun. Antonia. Reserva Biológica Sapitanduva, 25 Oct 1989, *K.U. Kramer and G. Hatschbach 10810* (F)*. Mun. Céu Azul, Rio Floriano, Parque Nacional do Iguaçu, 19 Apr 2004, *O.S. Ribas et al. 6123* (SPF). Santa Catarina: Bom Retiro Riosinho, 4 Dec 1948, *Reitz 2787* (RB).

Selaginella pallescens (C. Presl) Spring in Mart., Fl. Bras. 1(2):132. 1840.
Lycopodium pallescens C. Presl, Reliq. Haenk. 1:79. 1825. Fig. 3 G–I.

Megaspores globose to subtriangular, white, 280–300 μm in diameter, heteropolar, curvaturae perfectae; trilete aperture conspicuous, simple, wider than high, 190 μm in diameter; laesurae extending to 7/8 of the distance to the equator, pronounced, straight, smooth, not wing-like, increasing towards the pole, without complex mass, scabrate, not foveolate; sculpture in the center, close to the laesurae differs to the sculpture along the edge. Reticulum (proximal faces) absent; macrosculpture (proximal faces) baculate, reticulate, perforate, not foveolate; microsculpture (proximal faces) micro-echinate, scabrate, micro-perforate. Reticulum (distal faces) with 7–8 muri/100 μm , closed, irregular; muri 5–10 μm high, 0–5 μm wide, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) echinate, reticulate, microsculpture (distal faces) micro-echinate, scabrate, micro-perforate.

Distribution and habitat.—*Selaginella pallescens* is distributed in Nicaragua, Costa Rica, Mexico, North Colombia, Venezuela, Belize, El Salvador, Honduras, Surinam, and recorded for Brazil in the states: Rio de Janeiro, São Paulo, and Santa Catarina. It grows in forests and near forest edges in humus layers on mesic clay and shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo, culture Jardim Botânico de São Paulo, 12 Nov 1935, *F.C. Hoehne s.n.* (SP, SPF)*. Rio de Janeiro, Petropolis [23°30'18"S, 43°10'43"W], 10 Feb 2007, *F. Leitão 310* (RB). Rio de

Janeiro, Teresópolis [22°27'6"S, 42°59'22"W], 23 Sep 2011, *A. Lobão et al.* 1672 (RB).

Selaginella plana (Desv. ex Poir.) Hieron. in Engl. and Prantl, Nat. Pflanzenfam. 1(4):703. 1901. *Lycopodium planum* Desv. ex Poir. Lam. Encycl. suppl. 3:554. 1814. Fig. 3 J–L.

Megaspores globose to subtriangular, white, 210–235 μm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture simple, approximate wide as high, 135 μm in diameter; laesurae extending to 3/4 of the distance to the equator, straight, coarse, not wing-like, the whole length the same height, without complex mass, scabrate, verrucate micro-perforate, not foveolate; sculpture in the center, close to the laesurae differs to the sculpture along the edge. Reticulum (proximal faces) absent; macrosculpture (proximal faces) clavate, baculate, gemmate, rugulate, not foveolate; microsculpture (proximal faces) micro-gemmate, micro-verrucate, scabrate, micro-perforate. Reticulum (distal faces) absent; macrosculpture (distal faces) clavate, baculate, gemmate, rugulate, not foveolate; microsculpture (distal faces) scabrate, micro-perforate.

Distribution and habitat.—*Selaginella plana* is native to peninsular Malaysia, Sumatra, Java, Timor, Ambon, Banda, Ceram, Bali, Flores, Buru, Sumbawa, Kei Island, Sulawesi, Ternate, Ceram, Saparua, Solor, and recorded in Brazil for the states: Amazonas, Pernambuco, Rio de Janeiro, and São Paulo. It grows in forests and near forest edges in humus layers on mesic clay and shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Boracéia, 23 Sep 1976, *R. Wells-Windisch and Ghillány* 583 (HB). Rio de Janeiro: Arboreto Jardim Botânico, 31 Mar 2003, *F.R. Simões* 21 (RB). Arboreto Jardim Botânico, 2 Jun 2004, *S.L. de Winter* 109 (RB). Guapimirim Trilha entre o Paraíso e o Jacarandá, Teresópolis, 20 Apr 2011, *R.A. Engelmann* 1734 (RB, NY, MBM). Rio Grande do Sul: Patria, 21 Oct 1879, *W. Ims*, s.n. (P).

Selaginella sellowii Hieron. Hedwigia 39:306. 1900. Fig. 4 A–C.

Megaspores globose, white, 285–305 μm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture simple, higher than wide, 240 μm in diameter; laesurae extending to 7/8 of the distance to the equator, pronounced, straight, smooth, not wing-like, increasing towards the pole, without complex mass, scabrate perforate, not foveolate. Reticulum (proximal faces) with 9–11 muri/100 μm , irregular, mostly open; muri 5–10 μm high, 0–10 μm wide, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) verrucate, rugulate, striate, psilate, not foveolate; microsculpture (proximal faces) micro-echinate, micro-verrucate, scabrate. Reticulum (distal faces) with 4–7 muri/100 μm , irregular closed; muri 5–15 μm high, 0–10 μm wide, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal

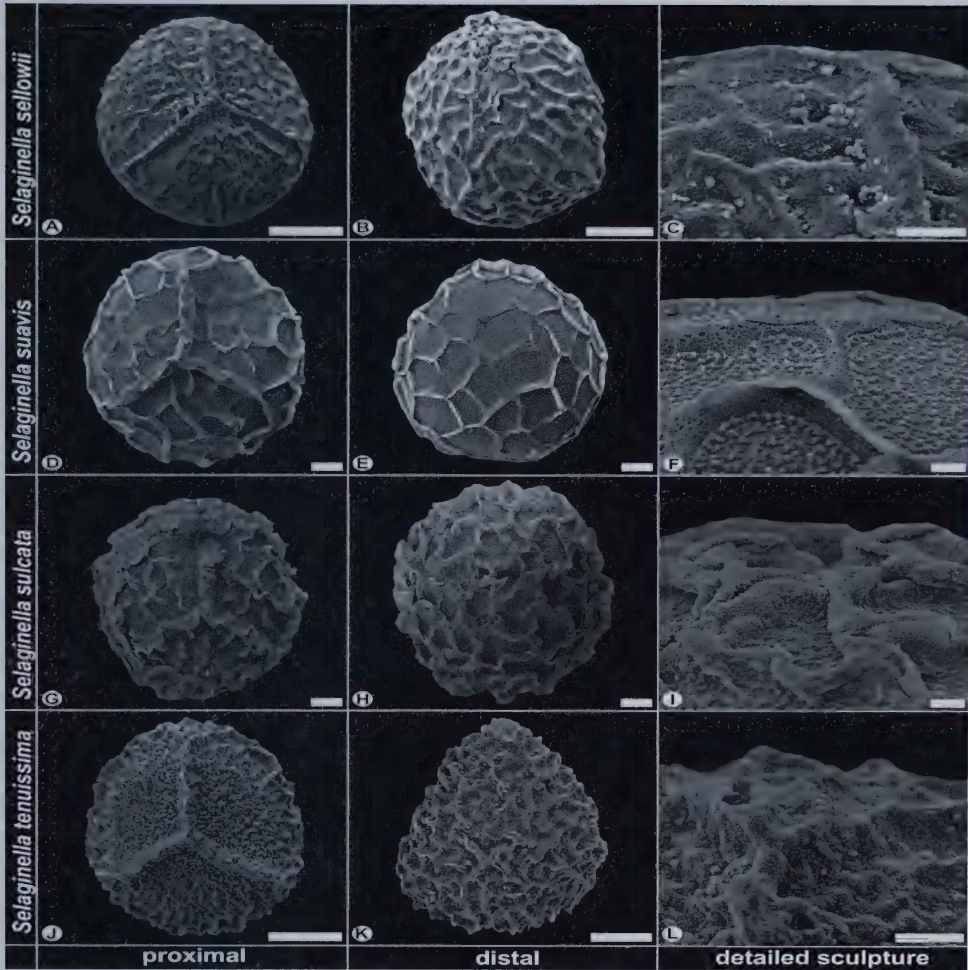


FIG. 4. Megaspores of *S. sellowii* (A–C), *S. suavis* (D–F), *S. sulcata* (G–I), and *S. tenuissima* (J–L). Scale bar proximal and distal faces 100µm, scale bar detailed sculptures 40µm.

faces) verrucate, rugulate, not foveolate; microsculpture (distal faces) micro-echinate, micro-verrucate, scabrate.

Distribution and habitat.—*Selaginella sellowii* is distributed in Bolivia, Ecuador, Paraguay, Peru, Uruguay, Argentina, Cuba, Venezuela, Colombia, Mexico, and recorded in Brazil for the states: Bahia, Mato Grosso, Espírito Santo, Rio de Janeiro, São Paulo, and Rio Grande do Sul. It grows on sandy humus soil and rock clefts in open sunny positions.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Cananéia, Ilha do Cardoso, 31 Aug 1977, *O. Yano* 825 (SP)*. Paranapiacaba, Estação Biológica, 28 Sep 1955, *O. Handro* 527 (SPF). Alto da Sera, Estação Biológica, 4 Sep 1931, *F. C. Hoehne* s.n. (SPF). [UTM: 23K 323247 7346459], 16 Nov 1997, *J.V. Coffani-Nunes et al.* 197 (SPF). Parque Estadual da Juréia, Peruíbe, Jan

1991, *M. Sobral and D. Attili 6625* (HRCB)*. [23°20'15.8"S, 45°09'00"W], 29 Oct 2001, *A. Salino et al. 7714* (HRCB)*. [23°26'56.6"S, 45°11'35.8"W], 1 Jan 2001, *A. Salino et al. 7788* (HRCB)*. Rio de Janeiro: Nas rochas atrás do Jardim, Cosme Velho 95, Nov 1941, *A. Lutz 1883* (R)*. Niterói Morro do Tucum, Itacoatiara - Parque Estadual da Serra da Tiririca, 7 Jul 2012, *L.P. Mauad 305* (RB). Itanahgá, Morro do Focinho do Cavalo, Estrada Barra da Tijuca em frente a Ponte Velha, 20 Oct 2010, *C.N. Fraga 3146* (RB). Alto da Boa Vista, 8 Oct 1966, *D. Sucre 1133* (RB). Niterói, Jurujuba, 24 Jun 1941, *s.c., s.n.* (SPF). Minas Gerais: [17°51'0.4"S, 57°35'00.4"W]. 23 Nov 2001, *G.A. Damasceno et al. 3047* (HRCB)*. Espírito Santo: Vale de Pancas, ca. 21 km a nordeste de Colatina, beira da estrada e em pedras. 8 Sep 1977, *G.J. Sheperd et al. 5854* (F)*. Entre Colatina e Patrimônio 15, 4 Dec 1971, *A. \P. Duarte 13987* (RB). Rio Grande do Sul: Alegrete, Dec 1981, *J. R. Stehmann and M. Sobral s.n.* (ICN). Jaguari, 1 Oct 1981, *R. Bueno s.n.* (ICN). Lavras do Sul, 14 Jul 1994, *C. Kazmirczak 110* (ICN). Bahia: vicinity of Machado Portello, 19 Jun 1915, *J. \N. Rose 19937* (RB).

Selaginella suavis (Spring) Spring, Bull. Acad. Roy. Sci. Brux. 10:229. 1843. Fig. 4 D–F.

Megaspores globose, white, 750–890 μm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture simple, higher than wide, 580 μm in diameter; *laesurae* extending to 7/8 of the distance to the equator, pronounced, straight, smooth, not wing-like, the whole length the same height, without complex mass, psilate, perforate, not foveolate. Reticulum (proximal faces) with 1 muri/100 μm , irregular, closed; muri 25–40 μm high, 15–20 μm wide, coarse, mostly acute; the sides of most muri sloping gradually to meet the underlying lumina; macrosculpture (proximal faces) echinate, striate, reticulate, rugulate, perforate, not foveolate; microsculpture (proximal faces) scabrate, micro-perforate. Reticulum (distal faces) with 1 muri/100 μm , irregular, closed; muri 15–25 μm high, 10–15 μm wide, coarse, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) echinate, reticulate, without additional ornamentation, not foveolate; microsculpture (distal faces) scabrate, micro-perforate.

Distribution and habitat.—*Selaginella suavis* is distributed in Peru and Brazil and recorded in Brazil for the states: Mato Grosso, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, and Santa Catarina. It grows in forests and near forest edges in humus layers on mesic clay.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Ilha do Cardoso, 7 Oct 1980, *E. Forero et al. 8586* (RB). [23°32'67"S, 45°02'72"W], 18 Feb 2004, *J. Prado et al. 1505* (RB, SP)*. Ubatuba, Lagoinha, Ruína dos Escravos, 14 Nov 1998, *R.Y. Hirai et al. 104* (HUEFS, SP*). Cananéia, Ilha do Cardoso, 5 Aug 1977, *O. Yano 799* (SP, MBM)*. Paraibuna, 09 Feb 1977, *T. Silva 38* (SP)*. Serra da Cantareira, Jun 1885, *A. Löfgren s.n.* (BM, SP*). Serra da Cantareira, 2 Apr 1932, *F.C. Hoehne s.n.* (SPF). Cananéia, Parque Estadual Ilha do

Cardoso, 8 Jan 1999, *C. Kozera et al.* 902 (MBM, UPCB)*. Mun. Ubatuba, Sertão da Quina, 7 Sep. 1998, *O.S. Ribas and V.A. O. de Dittrich* 2697 (MBM). Rio de Janeiro: Cascade Puisay, 5 Aug 1870, *Simard* 4488 (F)*. Serra do Itatiaia, Mont Serrat, 15 Jun 1930, *A.C. Brade* 10048 (R)*. Itatiaia, Oct 1913, *A. Lutz* 561 (R)*. Linha Auxiliar, Nov 1916, *A. Lutz* 842 (R)*. Itatiaia Estrada Maromba, May 1950, *A.C. Brade* 20289 (RB). Mangaratiba Rio das Pedras, Reserva Ecológica de Rio das Pedras, 30 Nov 1996, *L. Sylvestre* 1248 (RB). Mangaratiba Reserva Ecológica Rio das Pedras, trilha para o Cambucá, 27 May 1998, *C.M. Mynssen* 212 (RB, SP)*. Minas Gerais: Coronel Pacheco Fazenda da Companhia, 20 Aug 1944, *E.P. Heringer* 1522 (RB). Descoberto Reserva Biológica da Represa do Grama, 21 Apr 2001, *B.C. Campos et al.* 270 (RB, CESJ). Coronel Pacheco Estação Exp. de Café, 12 Dec 1940, *E.P. Heringer* 469 (RB). Espírito Santo: southeast slope of Serra do Caparaó, 30 Nov 1929, *Y. Mexia* 4072 (F)*. Cariacica Localidade de Alegre, trilha do Pau Oco, 15 Feb 2008, *P.H. Labiak et al.* 4626 (RB, MBML, CEPEC, UPCB*). [20°19'17"S, 40°31'10"W], 4 May 2008, *A. M.A. Amorim et al.* 7329 (CEPEC, MBML, RB, UPCB*).

Selaginella sulcata (Desv. ex Poir.) Spring ex Mart. Flora 2:126. 1837.

Lycopodium sulcatum Desv. ex Poir. in Lam. Encycl. suppl. 3:549. 1814.

Fig. 4 G–I.

Megaspores globose, beige, 560–680 μm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture higher than wide, with thickened ends, 440 μm in diameter; laesurae extending to 2/3 of the distance to the equator, indistinct, smooth, sinuous, not wing-like, the whole length the same height, without complex mass, scabrate, not foveolate. Reticulum (proximal faces) with 2 muri/100 μm , irregular, mostly open; muri 20–45 μm high, 15–35 μm wide, coarse, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) baculate, reticulate, rugulate, striate, not foveolate; microsculpture (proximal faces) micro-clavate, scabrate. Reticulum (distal faces) with 2 muri/100 μm , irregular, closed; muri 15–25 μm high, 5–25 μm wide, porous, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) baculate, rugulate, perforate, without additional ornamentation, not foveolate; microsculpture (distal faces) scabrate, micro-perforate.

Distribution and habitat.—*Selaginella sulcata* is distributed in Bolivia, Paraguay, British Guyana, Surinam, French Guiana, Argentina, Ecuador, and Brazil and is recorded in Brazil for the states: Ceará, Pará, Amazonas, Paraíba, Pernambuco, Bahia, Mato Grosso, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul. It grows in forests and near forest edges in humus layers on mesic clay and shadowed rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Serra da Mantiqueira, 16 Mar 1939, *J.G. Kuhlmann and A. Gehrt s.n.* (SP, SPF)*. Ipiranga, Mato do Governo, Jun 1910, *H. Luederwaldt* 1659 (BM, SP*, SPF). Iguape, Estação

Ecológica Juréia-Itatins, Serra da Juréia, 28-31 May 1996, *P.H. Labiak et al.* 324 (SP)*. Peruíbe, Estação Ecológica da Juréia, Arpoador, trilha do Imperador, 7 Jan 1999, *J. Prado et al.* 979 (SP)*. Teodoro Sampaio, Pontal do Paranapanema, Reserva Florestal do Morro do Diabo, Rio Paranapanema, 10 Sep 1985, *P.G. Windisch* 4279 (ICN). Serra do Mar, above Ubatuba, 21 Aug 1976, *P.H. Davis* 59813 (MBM). Mun. Ubatuba, Picinguaba, 11 Mar 1989, *A. Furlan et al.* 721 (HRCB)*. [24°12'-24°15'S, 48°03'-48°06'W], 13 Nov 2001, *V.A. de O. Dittrich* 1022 (HRCB)*. Rio de Janeiro: Theresopolis, Fazenda Cumary à Cachoeira Ferroz, Jan 1944, *B. Lutz* 2080 (R)*. Corcovado, s.d., s.c. (R)*. Vertente N.W. da Serra da Piaba, 17 Jul 1970, *D. Sucre* 7051 (RB). Minas Gerais: Marliéria Parque Florestal Estadual do Rio Doce, 18 May 1982, *H.P. Bautista* 568 (RB). Mun. de Marliéria, Parque Florestal Estadual do Rio Doce, 18 May 1982, *H.B. Bautista* 568 (MBM). Paraná: [25°46'37.9"S, 49°7'23.4"W], 24 Mar 2011, *P. Fiaschi et al.* 3590 (SPF). [25°49'43.5"S, 50°50'38.6"W], 20 Apr 2005, *R. Wasum* 2823 (ICN). Santa Catharina: s.l., 4 Mar 1906, *J.P. Schmalz* 152 (F)*. Rio Grande do Sul: Mun. De Rio Bonito, Biaçanã, Fazenda das Bachaena, 27 Apr 1977, *P. Lachlette* 299, 425 (R)*. Parque Est. Turvo, Tenente Portela, 11 Jan 1982, *R. Bueno s.n.* (ICN). Pernambuco: [08°59'27.3"S, 36°07'23.9"W], 28 Apr 2001, *M.R. Pietrobom et al.* 5124 (MBM). Goiás: [16°31'56.4"S, 49°06'50.2"W], 31 Mar 2005, *R.C. Mendonça et al.* 5924 (RB).

Selaginella tenuissima Fée, Cr. vasc. Br. 2:98. t. 108, f. 1. 1873. Fig. 4 J–L.

Megaspores globose to subtriangular, white, 255–330 μm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture simple, approximate wide as high, 235 μm in diameter; *laesurae* extending to 3/4 of the distance to the equator, pronounced, coarse, straight, not wing-like, the whole length the same height, without complex mass, gemmate, scabrate, not foveolate; sculpture in the center, close to the *laesurae* differs to the sculpture along the edge. Reticulum (proximal faces) with 45 muri/100 μm , irregular, approximate closed; muri 10–30 μm high, 0–10 μm wide, mostly acute; the sides of most muri sloping gradually to meet the underlying lumina; macrosculpture (proximal faces) clavate, gemmate, verrucate, rugulate-cristate, not foveolate; microsculpture (proximal faces) micro-gemmate, scabrate, micro-rugulate-cristate, micro-perforate. Reticulum (distal faces) with 4–5 muri/100 μm , irregular, mostly open; muri 5–20 μm high, 0–10 μm wide, coarse, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) clavate, rugulate-cristate, not foveolate; microsculpture (distal faces) micro-gemmate, scabrate, micro-perforate.

Distribution and habitat.—*Selaginella tenuissima* is endemic to Brazil and recorded for the Brazilian states: Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul. It grows in sandy humus, on mesic clay, and rocks.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Campos da Bocaina, Campos de Cunha-Macacos, 4 Mar 1992, *P.G. Windisch* 6824a (ICN, SJRP).

Jaraguá, 24 Mar 1912, *A.C. Brade 5137* (NY). Rio de Janeiro: Parque Nacional de Itatiaia, trilha dos Três Picos, 2 Jun 2006, *C.M. Mynssen 965* (RB). Itatiaia Planalto, próximo ao topo das Prateleiras, 30 Apr 1998, *K.T. Ribeiro 303* (RB). Serra dos Órgãos, Campo das Antas, 4 Sep 1950, *A.C. Brade 20507* (RB). Teresópolis Parque Nacional da Serra dos Órgãos, Trilha entre a Pedra do Sino e o Campo das Antas, Descida do grotão entre a Pedra do Sino/Garração e Pedra da Baleia, 13 Sep 2007, *L. Sylvestre et al. 2123* (RB, RBR, PNSO). [22°22'48"S, 44°39'47"W], 11 Jan 2008, *P.H. Labiak et al. 4458* (UPCB)*. Minas Gerais: Serra do Papagaio, in campis humidis, Nov 1897, *A.A. Silveira s.n.* (R)*. Passa Quatro Itaguaré, 9 May 1948, *A.C. Brade and A. Silva 19040* (RB). [21°40'44"S, 43°52'59"W], 11 Aug 2005, *C.M. Mynssen et al. 856* (RB, SP*, NY). Camanducia, Vila Monte Verde, Serra da Mantiqueira, 22 Mar 1991, *P.G. Windisch 6069* (ICN). [18°25'56.24"S, 43°28'01.03"W], 8 Feb 2013, *D.S. Bauer et al. 18* (HRCB)*. Paraná: Guaratuba, Parque Nacional Saint-Hilaire, Lange, Serra da Prata, 17 May 2006, *F.B. Matos et al. 1161* (UPCB)*. Parque Estadual Pico Paraná, 22 May 2008, *F. Meyer et al. 251* (UPCB)*. Idem, 29 Sep 2007, *M. Reginato et al. 23* (UPCB)*. Idem, *F. Marinero and J.B.S. Pereira 350* (UPCB)*.

Selaginella valida Alston, J. Bot. Lond. 70:281. 1932. Fig. 5 A–C.

Megaspores globose to subtriangular, white, 720–820 μm in diameter, heteropolar, indistinct curvaturae perfectae; trilete aperture conspicuous, simple, higher than wide, 470 μm in diameter; laesurae extending to 7/8 of the distance to the equator, pronounced, coarse, slightly sinuous, not wing-like, descending towards the pole, without complex mass, psilate, perforate, not foveolate. Reticulum (proximal faces) with 0.8–1 muri/100 μm , irregular, mostly open; muri 30–45 μm high, 10–15 μm wide, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) clavate, gemmate, verrucate, rugulate, perforate, not foveolate; microsculpture (proximal faces) micro-gemmate, micro-verrucate, scabrate, micro-perforate. Reticulum (distal faces) with 0.8–1 muri/100 μm , irregular, closed; muri 25–55 μm high, 15–50 μm wide, coarse, mostly acute; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) clavate, gemmate, verrucate, rugulate, perforate, without additional ornamentation, not foveolate; microsculpture (distal faces) micro-gemmate, micro-verrucate, scabrate, micro-perforate.

Distribution and habitat.—*Selaginella valida* is endemic to Brazil and recorded for the states: Rio de Janeiro, Paraná, Espírito Santo, and São Paulo. It grows in forests and near forest edges in humus layers on mesic clay.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. s.l., 1907, *A.F.M. Glaziou s.n.* (F)*. São Paulo, s.l., 1936, *A.H.G. Alston 317*, paratype (R)*. São Paulo, Santos, s.d., *C.W.H. Mosén 3812* (R)*. São Paulo, Mongaguá Pequena trilha que dá acesso a uma das cachoeiras da região, 2 Aug 1997, *F.P.F. Athayde 231* (RB, SP)*. São Paulo, Vila Atlântica, 25 Nov 1949, *A.B. Joly 826* (RB). Paraná, Antonina Reversa Natural Rio Cachoeira, Trilha do Corvo, 1 May 2005, *F.B. Matos et al.*

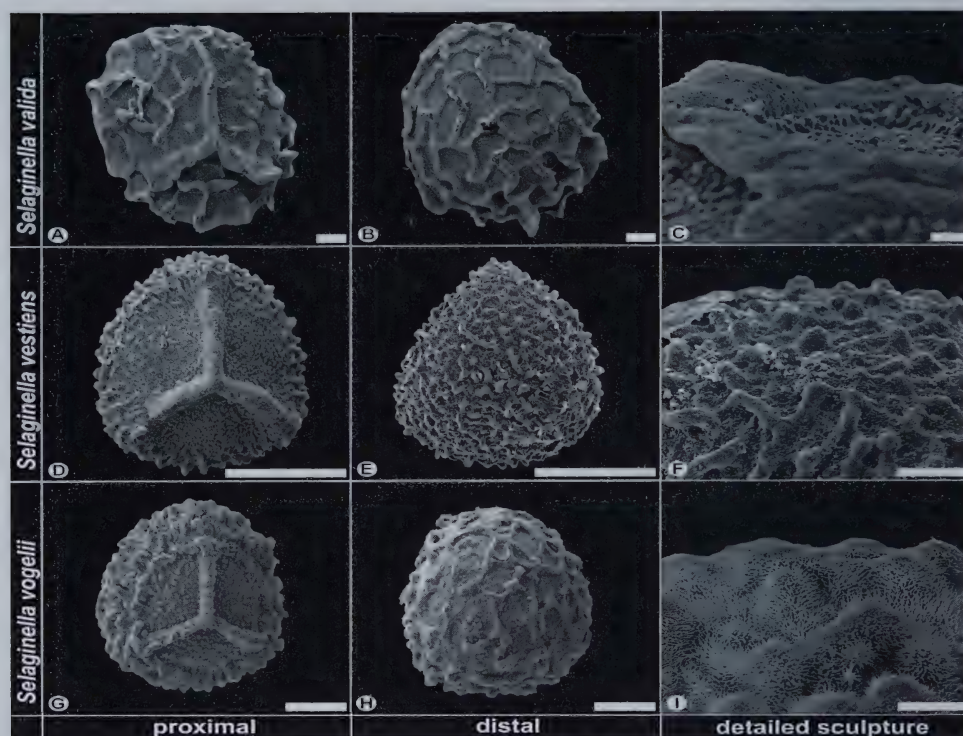


FIG. 5. Megaspores of *S. valida* (A–C), *S. vestiens* (D–F), and *S. vogelii* (G–I). Scale bar proximal and distal faces 100µm, scale bar detailed sculptures 40µm.

557 (RB, MBM, UPCB)*. Paraná, Antonina Reserva Natural Rio Cachoeira, Trilha do Corvo, 6 Oct 2005, *F.B. Matos and P.B. Schwartsbund* 826 (RB, UPCB, MBM)*. Rio de Janeiro, [22°24'09"S, 42°44'12"W], 26 Nov 2010, *R.A. Engelmann et al.* 1257 (RB, MBM)*. São Paulo, Sete Barras, Fazenda Intervalles, 12 May 1993, *S. Aragaki et al.* 69 (SP)*. São Paulo, Iguape, Estação Ecológica de Juréia, 15 Aug 1990, *J. Prado et al.* 327 (SP)*. São Paulo, Itanhaém, Vila Atlântica, Serra do Mar, 13 Jul 1956, *M. Kuhlmann* 3904 (SP, SPF)*. São Paulo, Sete Barras: Fazenda Intervalles, Saibadela, Trilha do Araçá ou Trilha no. 2, 6 Jul 1992, *R. Mello-Silva et al.* 573 (SPF). Espírito Santo, Santa Teresa: Nova Valsugana, ES-080- estrada não-pavimentada para Santa Leopoldina, ca. 1 km do entroncamento com a ES-261, 20 Jan 2011, *J.R. Pirani et al.* 6136 (SPF). Paraná, Rio Maê Catira, Mun. Morretes, 14 Dec 1969, *G. Hatschbach* 23231 (MBM, UPCB)*. Paraná, Recanto Engenheiro Lacerda, Mun. Morretes, 21 Jan 1999, *F.R.V. Pacheco et al.* 1505 (MBM). Paraná, Antonina Reserva Natural Rio Cachoeira, Trilha do Corvo, 1 May 2005, *F.B. Matos et al.* 557 (UPCB)*. São Paulo, Parque Estadual Intervalles, Base de Saibadela, Mun. Sete Barras, 14 Dec 2000, *V.A. de O. Dittrich and C. von Allmen* 823 (HRCB)*. São Paulo, [24°12'–24°15'S, 48°03'–48°06'W], 13 Nov 2001, *V.A. de O. Dittrich* 990 (HRCB)*.

Selaginella vestiens Baker, J. Bot. Lond. 21:97. 1883. Fig. 5 D–F.

Megaspores globose, white, 170–230 μm in diameter, heteropolar, without *curvaturae perfectae*; trilete aperture simple, approximate wide as high, 130 μm in diameter; *laesurae* extending to 3/4 of the distance to the equator, pronounced, straight, smooth, not wing-like, increasing towards the pole, without complex mass, scabrate, not foveolate; sculpture in the center, close to the *laesurae* differs to the sculpture along the edge. Reticulum (proximal faces) with 3–6 muri/100 μm , irregular, mostly open; muri 5–10 μm high, 5–10 μm wide, porous, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (proximal faces) clavate, baculate, rugulate, perforate, without additional ornamentation, not foveolate; microsculpture (proximal faces) micro-gemmate, scabrate, micro-perforate. Reticulum (distal faces) with 4–7 muri/100 μm , irregular, mostly open; muri 5–10 μm high, 5–10 μm wide, coarse, mostly blunt; the sides of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) reticulate, rugulate, without additional ornamentation, not foveolate; microsculpture (distal faces) micro-gemmate, scabrate, micro-perforate.

Distribution and habitat.—*Selaginella vestiens* is endemic to Brazil and recorded for the states Minas Gerais and São Paulo. It grows on sandy humus soil and rock clefts.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: [20°11'50"S, 47°25'10"W], 18 Apr 2003, *D. Sasaki et al.* 434 (SPF). Minas Gerais: Serra do Espinhaço, ca. 18 km East of Diamantina, 20 Mar 1970, *H.S. Irwin et al.* 27953 (F)*. Minas Gerais, [21°42'09"S, 43°52'18"W], 9 Aug 2005, *C.M. Mynssen et al.* 798 (RB). São Sebastião do Paraíso Baú, 26 Apr 1945, *A.C. Brade* 17953 (RB). Mun. Santana do Riacho. Serra do Cipó: km 125 da Rodovia Belo Horizonte, Conceição do Mato Dentro, 26 Mar 1991, *J.R. Pirani et al.* CSFS 12102 (SPF). [19°18'S, 43°34'W], 24 Mar 1989, *J.R. Pirani and R. Mello-Silva* 11307 (SPF, CSFS). [18°21'07"S, 43°40'52"W], 28 Feb 1998, *R.C. Forzza et al.* 4041 (SPF). [18°25'57.5"S, 43°28'30.9"W], 5 Feb 2013, *A. I. Coan et al.* 197 (HRCB)*.

Selaginella vogelii Spring, Mem. Acad. Sci. Belg. 24:170, n. 111. 1850. Fig. 5 G–I.

Megaspores globose, white, 355–385 μm in diameter, heteropolar, *curvaturae perfectae*; trilete aperture higher than wide, 240 μm in diameter; *laesurae* extending to 3/4 of the distance to the equator, pronounced, straight, coarse, not wing-like, descending towards the pole, with thickened ends, without complex mass, clavate; gemmate, not foveolate; sculpture in the center, close to the *laesurae* differs to the sculpture along the edge. Reticulum (proximal faces) absent; macrosculpture (proximal faces) clavate, gemmate, rugulate-cristate, not foveolate; microsculpture (proximal faces) micro-echinate, scabrate, micro-reticulate-cristate. Reticulum (distal faces) with 3 muri/100 μm , closed, irregular; muri 10–20 μm high, 5–10 μm wide, porous, mostly blunt; the sides

of most muri meeting the underlying lumina at a nearly right angle; macrosculpture (distal faces) verrucate, rugulate, not foveolate; microsculpture (distal faces) micro-echinate, micro-gemmate, micro-rugulate-cristate.

Distribution and habitat.—*Selaginella vogelii* is native to Bioko Island (Fernando Pó), Angola, Gabon, Ghana, Guinea, Sierra Leone, Zaire, Liberia, Ivory Coast, Benin, Nigeria, Cameroon, Equatorial Guinea, Congo, Tanzania, Kenya, Zanzibar, Madagascar and, recorded in Brazil for the states: Rio de Janeiro and São Paulo. It grows in forests and near forest edges in humus layers on mesic clay.

ADDITIONAL SPECIMENS EXAMINED.—BRAZIL. São Paulo: Brotas, Fazenda Santa Elisa, 8 Sep 1991, A. Salino 1045 (UEC). Rio de Janeiro: Cultivada no Jardim Botânico, May 1937, *Brade s.n.* (RB). Guapimirim Parque Nacional da Serra dos Órgãos, Sede Guapimirim, 3 Dec 2004, R.A. Engelmann 116 (RB). [22°29'38.2"S, 43°00'04.9"W], 15 Apr 2011, J.A. Lombardi *et al.* 8410 (RB, HRCB)*. Arboreto Jardim Botânico, 13 Feb 2003, F.R. Simões 3 (RB). [22°26'56"S, 42°59'06"W], 13 Jan 2008, P.H. Labiak *et al.* 4474 (MBM, UPCB)*.

DISCUSSION

In this study, we compared the megaspore sizes of nine *Selaginella* species that had been measured in previous studies by Korall and Taylor (2006), Lorscheitter *et al.* (2006), and Taylor (1989) (Table 2). The megaspores of the investigated specimens of *S. kraussiana*, *S. suavis*, and *S. sulcata* showed a similar size range compared to the previous studies (Table 2). However, in our investigations *S. marginata*, *S. microphylla*, *S. muscosa*, *S. pallescens*, *S. plana*, and *S. sellowii* showed minor differences in megaspore size (Table 2). The different size ranges in different publications suggests that it is likely that the real size range has not previously been detected, and the measured size range in a single publication is usually too narrow. The degree of variability within species is difficult to detect if the sampling of megaspores per species is limited or if all sampling originates from the same distribution area.

The 19 São Paulo species we studied include representatives of several subgenera of *Selaginella*. A current comprehensive investigation reveals key characters that distinguish several new superclades/subgenera, clades/sections, and subclades within the genus *Selaginella* (Zhou and Zhang 2015; Zhou *et al.* 2015a). We discuss how the characters of the species investigated here fit into the new phylogeny of *Selaginella*.

The newly investigated species confirm the general patterns of the megaspore surface that were observed for the subgenera and sections classified by Zhou and Zhang (2015) and Zhou *et al.* (2015a). Within the subgenus *Stachygynandrum* the species *S. contigua*, *S. flexuosa*, *S. macrostachya*, *S. mendoncae*, *S. valida*, and *S. vestiens* possess a reticulum, which supports the reticulate ornamentation of this subgenus presented by Zhou and Zhang (2015) and Zhou *et al.* (2015a). The subgenus *Ericetorum* with the section *Articulatae*, which are represented in our study by *S. decomposita* and *S. tenuissima*, also support the reticulate ornamentations by Zhou and Zhang

TABLE 2. Comparison of investigated megaspore size with megaspore size of previous studies.

| Species | Current study | Korall and Taylor 2006 | Taylor 1989 | Lorscheitter <i>et al.</i> 2005 |
|-----------------------|---------------|------------------------|-------------|---------------------------------|
| <i>S. kraussiana</i> | 600–650 µm | – | – | 612–621 µm |
| <i>S. marginata</i> | 590–650 µm | – | – | 470–539 µm |
| <i>S. microphylla</i> | 180–200 µm | – | – | 217–244 µm |
| <i>S. muscosa</i> | 250–290 µm | – | – | 325–316 µm |
| <i>S. pallescens</i> | 280–300 µm | 300–400 µm | 240 µm | – |
| <i>S. plana</i> | 210–235 µm | – | 270 µm | – |
| <i>S. sellowii</i> | 285–305 µm | – | – | 350–386 µm |
| <i>S. suavis</i> | 750–890 µm | 850–950 µm | – | – |
| <i>S. sulcata</i> | 560–680 µm | 500–600 µm | – | 500–598µm |

(2015) and Zhou *et al.* (2015a). In our study the subgenus *Pulvinella* is represented by *S. convoluta*. Here, we found verrucate ornamentations that were typical for megaspores of this subgenus (Zhou and Zhang 2015; Zhou *et al.* 2015a). Within the subgenus *Heterostachys* with the section *Oligomacroporangiatae* the species *S. vogelii* shows verrucate ornamentations, which supports the verrucate ornamentations of this subgenus and section defined by Zhou and Zhang (2015) and Zhou *et al.* (2015a).

We investigated *Selaginella sellowii* Hieron. (Fig. 4 A–C), which is part of subgenus *Tetragonostachys* (after Jermy 1986) or part of the Homoeophyllae clade/subgenus *Ericetorum* (Zhou *et al.* 2015a, Zhou and Zhang 2015). *Selaginella sellowii*, with its monomorphic leaves and decussate sporophylls, is placed within the Homeophyllae clade (Zhou *et al.* 2015a). Its megaspores exhibit interwoven reticulation on their proximal and distal faces (e.g., Korall and Taylor 2006; Zhao *et al.* 2006; Zhou *et al.* 2006; Zhou *et al.* 2015a; Zhou *et al.* 2015b). With respect to this feature, our results are in agreement with those of previous studies (e.g., Lorscheitter *et al.* 2005; Morbelli 1977; Morbelli *et al.* 2001; Taylor 1989; Tryon and Lugardon 1991). Additionally, we found small variability in the verrucate macrosculptures of *S. sellowii* (Fig. 4 A–C), in contrast to previous reports by Lorscheitter *et al.* (2015) and Morbelli *et al.* (2001). *Selaginella sellowii* could be a case of a widely distributed species (Hassler and Schmitt 2015) that exhibits intraspecific macrosculpture variability at the population level. Molecular investigations of *S. sellowii* populations across the range of the species are needed to clarify this issue.

Within the subgenus/superclade *Heterostachys* (Jermy 1986; Zhou *et al.* 2015a), *S. muscosa* Spring (Fig. 3 D–F) was included in our study. The majority of this superclade is comprised of Asian species. (Zhou *et al.* 2015a). The megaspore surfaces of the New World species are commonly tuberculate, verrucate, or papillate, with few reticulate structures (Zhou *et al.* 2015a). Our observations of *S. muscosa* (Fig. 3 D–F) agree with those made for the *Heterostachys* superclade by Zhou *et al.* (2015a) and other investigators (Lorscheitter *et al.* 2005; Morbelli 1977; Morbelli *et al.* 2001). Two other species of the *Heterostachys* superclade (Zhou *et al.* 2015a) in our study were *S. vogelii* Spring (Fig. 5 G–I) and *S. plana* (Desv. ex Poir.) Hieron. (Fig. 3 J–L). Within this

superclade, *S. vogelii* is inside the Oligomacrosporangiatæ clade, and because of its habit it is placed in the *S. pervillei* subclade (Zhou *et al.* 2015a). The clavate megaspore surface characters of *S. vogelii*, reported here for the first time (Fig. 5 G–I), are concordant with the structures of the *S. pervillei* subclade (Zhou *et al.* 2015a). Characteristics of *S. plana* (Fig. 3 J–L) are similarly in agreement with those of the *S. delicatula* subclade (Zhou *et al.* 2015a), and its megaspore surface characters are as reported in previous studies (Korall and Taylor 2006; Taylor 1989). Additional studies that combine molecular and morphological data will be critical in building support for these proposed clades.

Within the proposed Ericetorum superclade (Zhou *et al.* 2015a), we studied seven species. This superclade was represented by the subgenus *Stachygynandrum* (Jermy 1986) and the Articulatæ clade within this subgenus (Hieronymus 1901; Korall and Taylor 2006). The latter clade supports the definition of the Articulatæ with their vegetative features inside the genus *Selaginella*. Those features were swollen branches with dorsal rhizophores, and one megasporangium per strobilus (Zhou *et al.* 2015a; Zhou and Zhang 2015). In addition to the New World species, we investigated for the first time both *S. tenuissima* Feé (Fig. 4 J–L) and *S. decomposita* Spring (Fig. 1 G–I), which are associated with the *S. fragilis* subclade (Zhou *et al.* 2015a). Our observations of *S. suavis* Spring (Fig. 4 D–F) and *S. sulcata* (Desv. ex Pior.) Spring ex Mart. (Fig. 4 G–I) are in agreement with those of earlier studies (Lorscheitter *et al.* 2005; Morbelli *et al.* 2001; Korall and Taylor 2006). *Selaginella marginata* (Kunth) Spring (Fig. 2 G–I) is also associated with the *S. fragilis* subclade (Zhou *et al.* 2015a) on the basis of its megaspore surface characters, and our results agree with those of earlier studies of *S. marginata* (Lorscheitter *et al.* 2005; Hellwig 1969; Morbelli 1977; Morbelli *et al.* 2001). Moreover, the disparity with respect to the vascular bundles of both subclades could not be proven, because the taxa of the *S. fragilis* subclade (Zhou *et al.* 2015a) show more than one vascular bundle. In addition, our observations of the megaspore characters of *S. kraussiana* (G. Kunze) A. Braun (Fig. 2 A–C) are in accordance with other observations of the *S. kraussiana* subclade (Zhou *et al.* 2015a).

Within the Rosulatæ clade (Zhou *et al.* 2015b), *Selaginella convoluta* (Arn.) Spring (Fig. 1 D–F) was the only species in our study. *S. convoluta* was included in this clade by Baker (1884) because of its rosette, erect habitus, and up-curling branches (resurrection species), and Zhou *et al.* (2015a) established that the clade has a verrucate spore surface. In our study, this was verified (Fig. 1 D–F) and shown to be in accordance with observations of earlier studies (Morbelli 1977; Morbelli *et al.* 2001).

All other investigated species were related to the subgenus *Stachygynandrum* (Jermy 1986) and were placed within the Stachygynandrum superclade (Zhou *et al.* 2015a). *Selaginella pallescens* (C. Presl.) Spring (Fig. 3 G–I) has a special position in this clade because of its different growth forms (erect and rosette) and variable number of chromosomes ($2n = 20, 22$). Notwithstanding those facts, our investigations confirm the reticulate megaspore nature of the surface of *S. pallescens* (Fig. 3 G–I) reported in prior studies (Korall and Taylor 2006; Taylor 1989; Zhou *et al.* 2015a). We also examined for the first time the

megaspores of *Selaginella macrostachya* Spring (Fig. 2 D–F), *S. mendoncae* Hieron. (Fig. 2 J–L), *S. valida* Alston (Fig. 5 A–C), and *S. vestiens* Baker (Fig. 5 D–F). These species are within the Stachygynandrum superclade (Zhou *et al.* 2015a). On the basis of their reticulate megaspore surface and their distribution in the New World (Hassler and Schmitt 2015), these four species are members of the *S. anceps* clade (Zhou *et al.* 2015a), although further molecular investigations should be carried out to verify this group's monophyly. In addition, *S. flexuosa* Spring (Fig. 1 J–L) and *S. microphylla* (Kunth) Spring (Fig. 3 A–C) megaspores were analyzed. Both species are within the Stachygynandrum superclade (Zhou *et al.* 2015a) and show reticulate megaspore surfaces as reported in previous studies (Lorscheitter *et al.* 2005; Morbelli *et al.* 2001; Zhou *et al.* 2015a).

The investigated megaspores showed small a variability regarding the spore ornamentation. This small variability arises during the development of the exospore sculpture. At this time, the ornaments of the megaspore surface can be subject to change in shape and size. This process within *Selaginella* exospore sculpture is well documented as self-assembly development (Hemsley *et al.* 1994; 1996; 1998).

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Characterizing Quantitative Variation in the Glossopodia of Three Western North American *Isoëtes* Species

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ABSTRACT.—One of the challenges when working with the genus *Isoëtes* is the scarcity of in-depth comparative analyses of its morphological characters. This paucity of analyses relates in part to the long-held conclusion that most of the characteristics associated with the traits of the genus aside from spore morphology, are too variable to be informative. As a result, our understanding of morphological variability among species of *Isoëtes* is underdeveloped. Recent workers have attempted to address this deficiency by examining the glossopodia of the ligule, a structure unique among living taxa to *Isoëtes* and its sister genus, *Selaginella*. The glossopodium is embedded in the adaxial surface of the microphyll, and has shown evidence of interspecific variability in the few studies in which it has been examined. However, these prior works were limited to qualitative comparisons. The present study compares 3D reconstructions of the glossopodia of three species, *Isoëtes howellii*, *I. bolanderi* and *I. nuttallii*, using morphometric analyses in addition to more traditional qualitative comparisons. MANOVA of measurements obtained from 3D reconstructions of the glossopodia show significant differences in size while elliptical Fourier analysis of these reconstructions demonstrates differences in shape among the species. These analyses provide a repeatable, statistical framework for future work on the glossopodium and potentially other traits in *Isoëtes*.

KEY WORDS.—3D model, elliptical Fourier analysis, ligule, morphometric

Isoëtes L. is one of the two extant genera of heterosporous lycopods. Unlike seed plants, ferns, and horsetails, which are members of the trimerophyte lineage, the lycopods are members of the zosterophyll lineage. *Isoëtes* is cosmopolitan in distribution, occurring on all continents except Antarctica (Hoot *et al.* 2006). The genus includes ca. 300 described species, although there is evidence of a considerable number of undescribed species (Freund, unpublished data; Jung *et al.*, 2014;). Polyploidy is common in the genus, occurring in ca. 60–70% of all described species (Hoot *et al.* 2006). The plants are found in areas that are either temporarily or permanently wet, and can be found growing in aquatic (i.e., permanently submerged), terrestrial (i.e., non-submerged), or amphibious (i.e., temporarily submerged) habitats.

For botanists studying the genus, there has been a long-standing problem with finding informative characters. At the species level, features including leaf length, number, texture, color, stoma presence or absence, number of peripheral strands in the leaves, and velum coverage of the sporangia have been commonly applied (Pfeifer 1922). Delimiting infrageneric species

groups has been based on either habitat (Engelmann, 1882; Motelay and Vendryes, 1883) or surface ornamentation of the megaspores (Pfeiffer, 1922). However, there is evidence from morphological and molecular data that these traits are homoplastic, appearing to represent repeated, convergent evolution of the same character state (Hickey, 1986b; Hoot *et al.*, 2006; Taylor and Hickey, 1992). In addition, questions have been raised concerning the reliability of the characters historically used for species identification (Kott and Britton, 1985; Small and Hickey, 2001; Yadav *et al.*, 2012).

There are several documented impediments to studying the morphology of the genus. One of the most basic issues is that many species, particularly those from the tropics, are poorly collected. In some cases, only the type specimens are available (Hickey, 1986a; Hickey *et al.*, 1989). Another difficulty is that most of the morphological studies in the genus have focused only on the megaspores, or the developmental anatomy or morphology of a single species (Bhambie, 1963; Budke *et al.* 2005; Campbell, 1891; Paolillo, 1963; Scott & Hill, 1900; Stokey, 1909), rather than multivariate comparisons among fully mature organs and structures between species.

Fortunately, several recent papers have sought to address this deficiency in informative morphological characters (Budke *et al.* 2005, Shaw and Hickey 2005). These studies have suggested that characteristics of the ligule might have utility both in delimiting species and developing phylogenetic hypotheses (Sharma and Singh, 1984; Shaw and Hickey, 2005).

Among extant taxa, the lycopod ligule is a structure unique to the heterosporous lycopoids (*Isoetes* and *Selaginella*), although it is also potentially present in a small number of extinct homosporous lycopods (Greirson and Bonamo, 1979; Pigg, 2001). Despite the long evolutionary history of this structure, its exact ecological and biological function is not well understood. In *Isoetes* the ligule is large, while those of *Selaginella* are proportionally much smaller and not as deeply embedded in the leaf (Horner *et al.* 1975; Pant *et al.* 2000). In *Isoetes*, the ligule is visible on the adaxial leaf base distal to the sporangium (Fig. 1a) and can be divided into three general regions (Fig. 1b). The first, and most easily examined, is the ephemeral, leaf-shaped external portion alternatively referred to as the tongue or simply, in the older literature, the ligule (Kott, 1980; Pfeiffer, 1922; Smith, 1900). This portion of the structure is known to be secretory, producing a complex mucilage of polysaccharides and polypeptides (Kristen *et al.*, 1982). The function of this exudate is unknown, but hypotheses have included protection and moistening of the young leaves (Sharma and Singh, 1984), an aid to nutrient transport (Kristen and Biedermann, 1981; Sharma and Bohra, 2002), or possibly as a vestigial structure once used for carnivory (Kristen *et al.*, 1982). However, as the full function of the ligule has not been studied, there is little evidence to support any of these proposed functions. Connecting the tongue to the embedded, internal part of the ligule is what has recently been termed the medimoles (L., *media* = middle, *moles* = shapeless mass; Shaw and Hickey, 2005), which has no known function other than to bridge

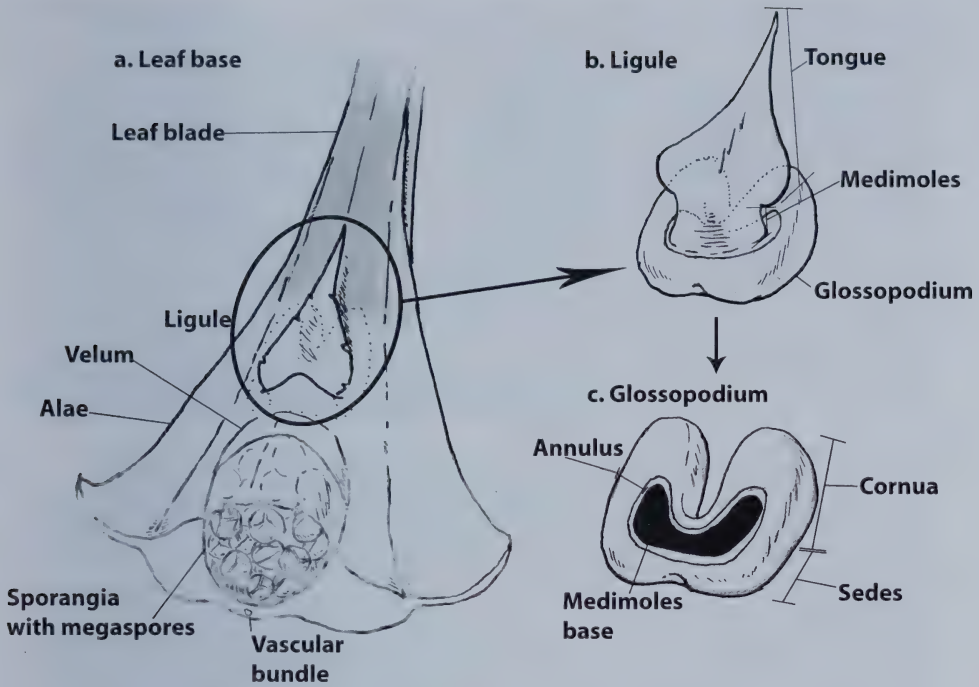


FIG. 1. The leaf base of *Isoetes*, including the structures of the leaf, ligule, and glossopodium. a. Leaf base structures. b. Ligule structures. c. Glossopodium structures. NB: Images are not to scale, and represent the average appearance of *Isoetes howellii*.

the two other portions of the ligule. Attached to the medimoles at a region I call the “medimoles base” is the glossopodium (Fig. 1c; L., *glosso* = tongue, *pod* = foot; pl. = glossopodia; Bhambie, 1963; Smith, 1900), which has a roughly “U” shaped outline. The distal portion of the glossopodium is composed of two distinctive projections called cornua (L., *cornua* = horns; singular = cornu; Bhambie, 1963; Smith, 1900), which rests upon a broad base that I refer to as the “sedes” (L., *sedes* = foundation). Additionally, running the circumference of the adaxial face of the glossopodium is a raised lip here referred to as the “annulus” (L., *annulus* = ring; pl. = annuli; Fig. 1c, Fig. 2 [adaxial face]). At the interface between the glossopodium and the leaf ground tissue is a layer of small, isodiametric cells that mark the leaf boundary. Smith (1900) refers to these as sheath cells, though Dunlop (1949) referred to them as endodermal cells due to the reputed presence of a Casparian strip, although I did not observe the latter. The sheath cells terminate at the inner limit of the annulus (Fig. 3).

Even without understanding the exact ecological and evolutionary function of the ligule, there is evidence of its presence in Isoëtopsida for millions of years (Pant *et al.*, 2000; Pigg, 1992; Retallack, 1997). Bhambie (1963) provided the first depiction of a fully characterized glossopodium from

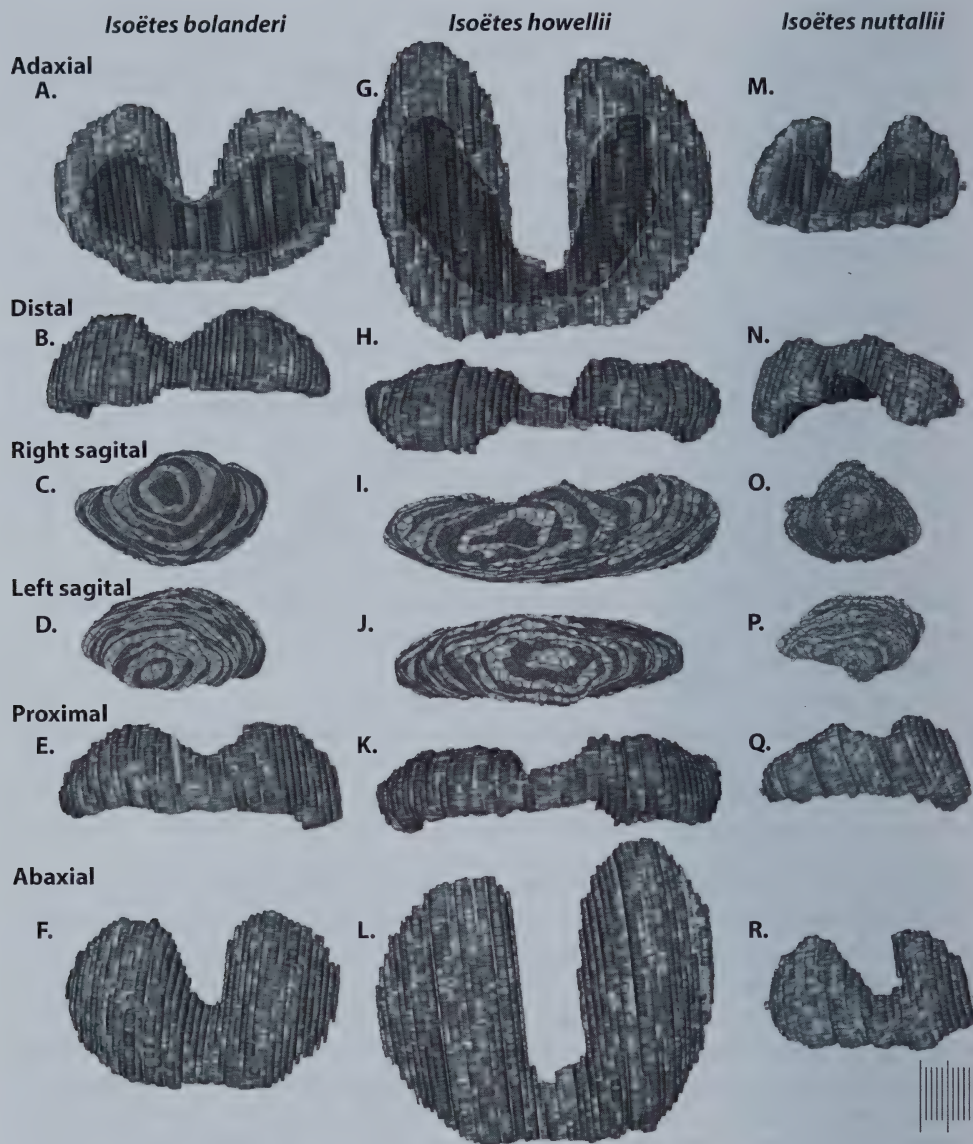


FIG. 2. Representative 3D reconstructions for each of the sampled species showing all six faces. Scale = 0.1mm with 0.01mm increments.

Isoetes coromandelina L.f. (a widespread species from Southeast Asia and northwestern Australia). Sharma and Singh (1984) later described the glossopodia of three Indian species, *I. coromandelina*, *I. rajasthanensis* Gena & Bhardwaja and *I. reticulata* Gena & Bhardwaja; however, their interpretations of *I. coromandelina* were quite different from those of Bhambie. Shaw and Hickey (2005) followed up on this work by examining

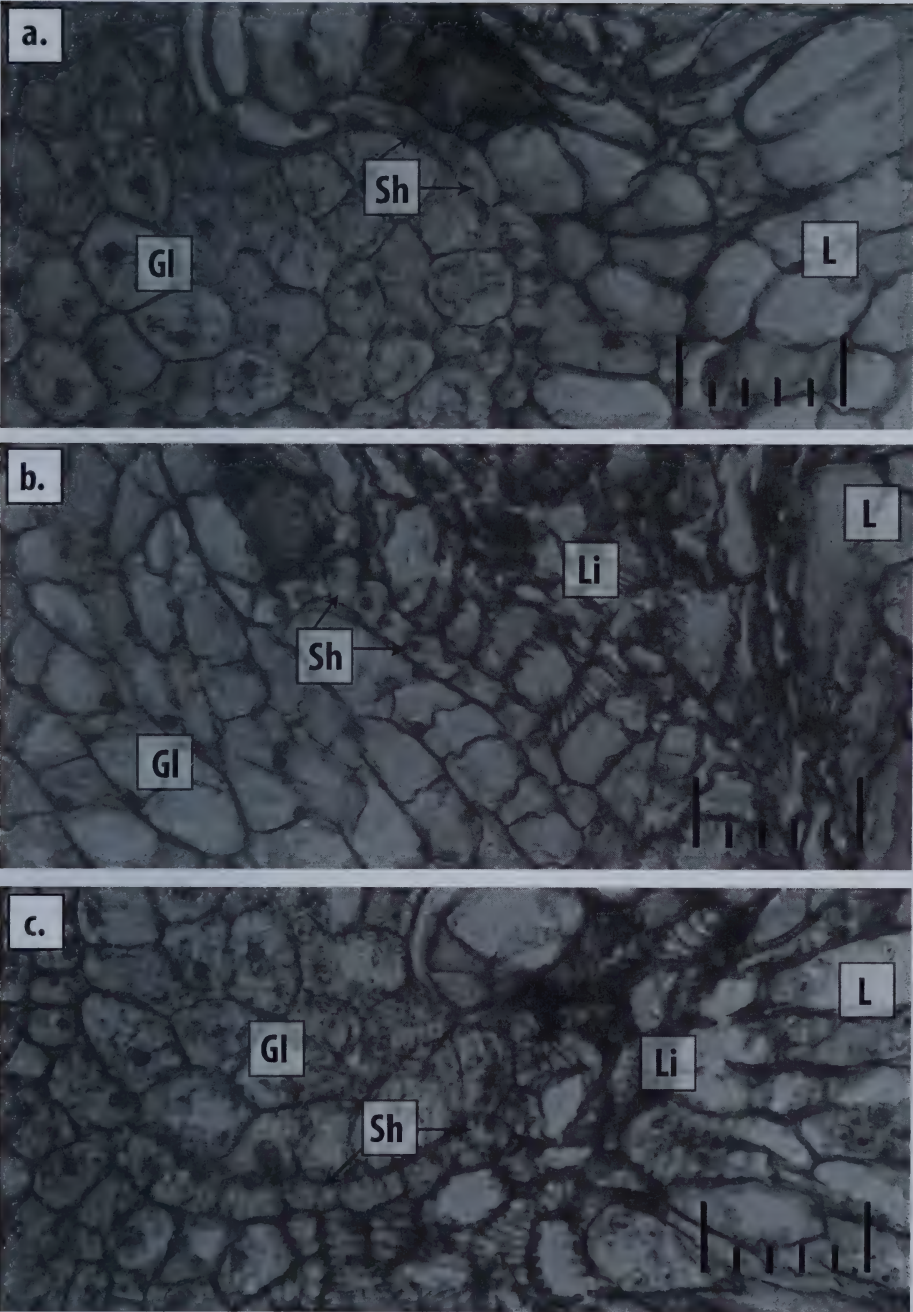


FIG. 3. Glossopodial (Gl), sheath (S), leaf (L) and lignified (Li) cells in sagittal sections of a) *I. bolanderi*, b) *I. howellii*, and c) *I. nuttallii*. Sheath cells are indicated with arrows. Scale = 0.05mm with 0.01mm increments.

three New World species, *I. melanopoda* J.Gay & Durieu, *I. virginica* N.Pfeiff., and *I. tennesseensis* Luebke & Budke, using 3D reconstructions in addition to histology to interpret the form of the glossopodia. These studies provided a wealth of new information on the shape of this structure, including its variability among species. However, these comparisons were strictly qualitative in nature.

The present study seeks to build and improve upon these prior studies by utilizing both qualitative and statistical analyses to characterize the glossopodia of three species of *Isoëtes* from the California Floristic Province. Though they are not endemic to the region, *Isoëtes bolanderi* Engelm., is an aquatic species found in lacustrine environments at mid to high elevations; *I. howellii* Engelm., is an amphibious species found in both vernal pools and lake margins at low to mid elevations; and *I. nuttallii* A.Br. is a terrestrial to amphibious species found in vernal pools and seeps, as well as on granite outcrops at a wide range of elevations.

Although the evolutionary relationships among the major clades of *Isoëtes* are well supported (Hoot *et al.*, 2006), there is still some ambiguity in the topology of the crown groups, with relationships differing depending on the DNA regions used (Freund, unpublished data; Hoot *et al.*, 2006). Two of the species chosen for this study, *I. bolanderi* and *I. howellii*, belong to a large clade found in North, Central, and South America. *Isoëtes nuttallii* is not part of this clade, but is instead in a smaller clade found only from southern British Columbia to northern Baja California (Hoot *et al.*, 2006, Freund, unpublished data). These three species are diploids, represent half of the described *Isoëtes* species diversity in the state of California (Taylor and Keeley, 2013), and provide a sampling across a range of habitats and phylogenetic relatedness. The two closely related species (*I. bolanderi* and *I. howellii*) are not sympatric. In contrast, *I. howellii* and *I. nuttallii*, which are distantly related, have overlapping ranges in California, though *I. nuttallii* tends to be found at higher elevations. All three species have similar tuberculate megaspore ornamentation; the megaspores otherwise differ mostly in size. If these three species can be distinguished using qualitative and quantitative morphological analyses of glossopodia, it would provide further support their comparative morphological value.

The goal of this study is to develop a methodology to assess the qualitative and quantitative differences of mature glossopodia among these three species, and to determine the value of this structure for distinguishing species and developing phylogenetic hypotheses. Two morphometric approaches are employed in addition to qualitative comparisons of the anatomy and morphology of the glossopodia. One approach is multivariate analysis of variance (MANOVA) to determine whether there are significant differences among the species based on the size of the glossopodium measured on computer-generated 3D reconstructions. A second approach uses elliptical Fourier analysis (EFA) on outlines generated from these same reconstructions to determine if there are significant differences in shape.

TABLE 1. Collection information. Elevations are in meters above sea level. Vouchers are deposited at RSA.

| Species | Site | Elev.
(m) | Coordinates | Collector & Col. # | Voucher |
|--------------------------|---------------------------------|--------------|----------------------------|--------------------|------------|
| <i>Isoëtes bolanderi</i> | Rock Creek Lake, CA | 2967 | 37.45379°N,
118.73845°W | Freund 8 | RSA 811640 |
| <i>Isoëtes howellii</i> | Fort Ord, CA – Twin Ponds East | 94 | 36.63255°N,
121.74371°W | Freund 125 | RSA 796365 |
| | Fort Ord, CA – Butterfly Valley | 144 | 36.64553°N,
121.74565°W | Freund 124 | RSA 796366 |
| <i>Isoëtes nuttallii</i> | Long Meadow Creek, CA | 1475 | 35.99265°N,
118.54942°W | Freund 170 | RSA 796376 |
| | HWY 89 South of Lake Tahoe, CA | 2014 | 38.80978°N,
120.01226°W | Freund 189 | RSA 811632 |
| | HWY 44 West of Susanville, CA | 1737 | 40.52171°N,
121.02367°W | Freund 193 | RSA811636 |

MATERIALS AND METHODS

Three plants of *I. bolanderi* were sampled from a single population at Rock Creek Lake in the Sierra Nevada Mountains, Inyo Co., CA. *Isoëtes howellii* plants were sampled from two populations at Fort Ord, Monterey Co., CA, with three plants taken from each population. A single individual of *I. nuttallii* was sampled from each of three populations in the northern, central and southern Sierra Nevada. Sampled plant materials were fixed in FPA (10:1:2, 95% ethyl alcohol: propionic acid: 37% formalin) upon collection. Vouchers were deposited at the Rancho Santa Ana Botanic Garden Herbarium (RSA; Table 1).

Three leaves were sampled from each individual except *I. howellii*, for which a single leaf was taken from each individual due to limited preserved materials. In all cases, the fertile leaves (either mega or microsporophylls) were taken from the outer layer of the rosette. Leaf bases containing the glossopodia were taken through an alcohol/xylene/paraffin embedding process, and sectioned at 10µm thickness in the dorsiventral (adaxial-abaxial), sagittal (left-right), and transverse (distal-proximal) planes using a Leica (Leica Microsystems, Inc., Buffalo Grove, IL, USA) rotary microtome (Ocampo and Columbus, 2010; Shaw and Hickey, 2005). Sections were mounted onto glass slides, stained using a modified Sharman staining series (Columbus, 1999; Ocampo and Columbus, 2010; Sharman, 1943), and permanently fixed with a cover slip using Cytoseal 60. Serial sections were photographed using a Leitz Laborlux D compound microscope and Insight 6 Firewire Digital Camera and Spot Advanced imaging software (Diagnostic Instruments, Sterling Heights, MI, USA). Each set of serial images was imported into Adobe Photoshop (Adobe Systems Incorporated, San Jose, CA, USA) using the “Load Files Into Stack” script. Images were aligned to the slices above and below by making the top section semi-transparent (50% opacity), to match up the

cell walls with the least amount of shift off 90° from the face of the cut. Once stacked, the images were edited to remove both leaf and sheath cells using the Lasso tool in conjunction with a Wacom tablet and stylus (Wacom Technology Corporation, Vancouver, WA, USA). All stacks were centered in the image frame, and were increased in contrast until the margin was easily visible, and a 1 mm scale bar was placed across the central slice. Edited images were exported from Photoshop using the “Export Layers Into Files” script, then re-imported and edited to remove the background using the Magic Eraser tool. Image stacks were then imported into ImageJ version 1.48q (Schneider *et al.*, 2012), and rendered as 3D objects, first by using the stacks > 3D Project tool to set a pixel separation of 10µm between the slices, then the 3D Viewer plugin (Schmid *et al.*, 2010) was used to render the stacks into 3D objects that could be rotated in three dimensional space. Three of the reconstructions were excluded from analysis due to aberrant, doubled ligules (see results).

A series of images were captured from each 3D object in the following orientations: 1) original orientation (to ensure correct positioning of the scale bar), 2) adaxial face, 3) abaxial face, 4) distal face, 5) proximal face, 6) right sagittal face and 7) left sagittal face (Fig. 2). Each of these images from a given sample and orientation was re-imported into ImageJ as a stack, and inter-landmark distances were measured on each after setting the scale using the scale bar embedded in the original orientation of the image. A total of 29 linear distances were measured on the abaxial, distal, proximal and sagittal faces by rotating the 3D reconstructions in space and making scaled measurements (Fig. 4, Table 2). Data were imported into R (R Development Core Team, 2008), using the R-studio (RStudio, 2014) interface, and log transformed to normalize the variance. MANOVA was run using the *MASS* (Venables and Ripley, 2002) and *psych* (Revelle, 2014) libraries. Homogeneity of variances was examined with the Bartlett, Fligner-Killeen, and Brown-Forsythe tests in *psych*. Each MANOVA was run on the set of distance measurements for each face using the Pillai statistic at $p=0.05$. TukeyHSD post hoc tests were run to determine which of the distances measured differed among species at the same p value.

The images used in the MANOVA measurement process were converted to gray-scale silhouettes using Adobe Photoshop and imported into R. Additionally, a silhouette of the base of the medimoles was also generated by tracing its outline on an image of the adaxial face. All silhouettes of a given face were analyzed using the *momocs* (Bonhomme *et al.*, 2014) and *scales* libraries (Wickham, 2014). The initial x-y coordinates were smoothed using a simple smoothing algorithm in *momocs* to eliminate noise due to the tiered appearance of the silhouettes, which resulted from the stacking process used to create the 3D objects. Elliptical Fourier analysis (EFA) was run on each set of boundaries, describing the shape by decomposing the coordinate data into a series of orthogonal harmonics and analyzing the coefficients. A principal components analysis was then run on these coefficients (without rescaling) to characterize shape variations and differences between species (Crampton

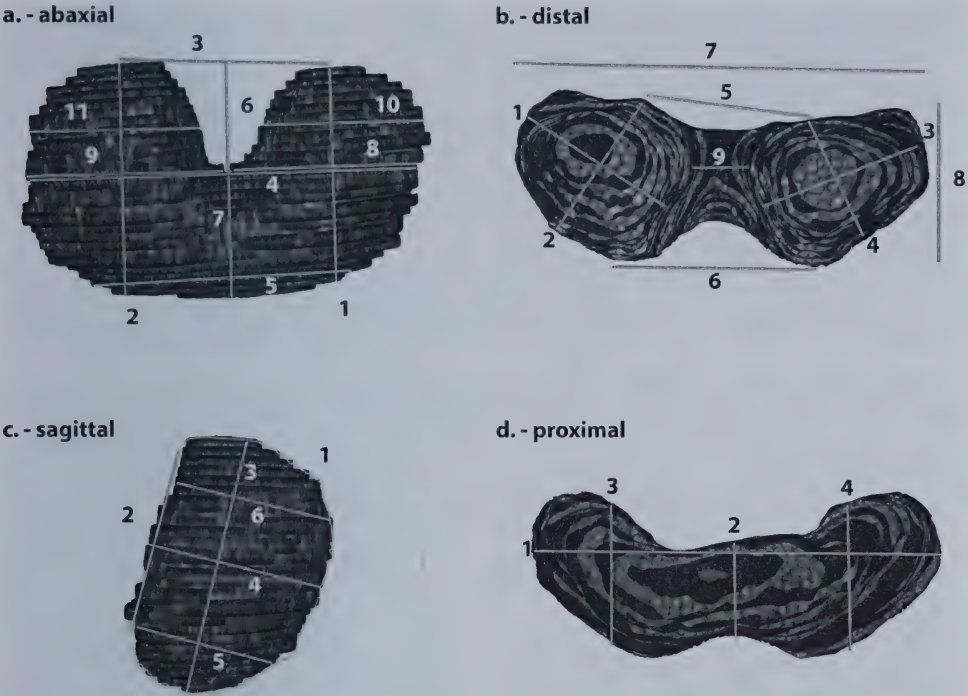


FIG. 4. Euclidean linear distance measurements taken for four projected slices through the glossopodium of *Isoetes*, used in MANOVA analysis.

1995, Neto *et al.* 2006). The supporting materials – including the R scripts used for basic descriptive statistics, the raw measurements, the raw images, and the appendices – are available on MorphoBank (www.morphobank.org/index.php/Projects/ProjectOverview/project_id/2075).

RESULTS

General anatomy of the glossopodia and surrounding leaf tissues.—The tissue of the ligule is immediately apparent in the sections, as are the adjacent sheath cells. These cells are noticeably smaller than the surrounding leaf tissue, and appear to have taken up both the safranin and orange G readily, staining them a distinctive reddish hue (Figs. 5–13). The cells of the leaf tissue are smaller and \pm isodiametric near the glossopodium, but become elongate as the leaf transitions from the leaf base to the blade.

Between the glossopodium and leaf ground tissues are the sheath cells (Dunlop, 1949; Smith, 1900), which serve as the boundary of the leaf tissue (Figs. 3; 5b–e; 6b–e; 7d–f; 8b–f; 9b–e; 10c–f; 11b–f; 12c–f; 13c–f). During the course of my examinations, I found no evidence of the Casparian strip that Dunlop (1949) reported. The cells of the glossopodium adjacent to the sheath

TABLE 2. Descriptions of the linear distances measured for MANOVA analysis shown in Fig. 4.

| Face | Number | Measurement description |
|-------------------------|---------|--|
| Abaxial | 1 & 2 | Maximum vertical height of the right (1) and left (2) cornu & sedes, measured at as close to 90° to horizontal as possible |
| | 3 | Distance between the apex of the cornua lobes |
| | 4 | Width of the sedes just below the saddle of the cornua |
| | 5 | Width of the sedes where it begins to transition into an upward curve |
| | 6 | Depth of the gap between the cornua |
| | 7 | Height of the midpoint of the sedes |
| | 8 & 9 | Width of the base of the right (8) and left (9) cornu |
| | 10 & 11 | Width of the cornu at the mean distance from the base to the apex of the left (10) and right (11) cornu |
| Distal | 1 & 3 | Maximum width of the left (1) and right (3) cornu |
| | 2 & 4 | Width of the left (2) and right (4) cornu at 90° to measures 1 and 3 |
| | 5 | Distance between the midpoints of the cornua on the adaxial face |
| | 6 | Distance between the most abaxial portions of the glossopodia |
| | 7 | Width of the glossopodia |
| | 8 | Height of the glossopodia |
| | 9 | Width of the gap between the cornua where the lobes are most equidistant from one another |
| Proximal | 1 | Maximum width of the sedes |
| | 2 | Width of the sedes at 90° to the midpoint of 1 |
| | 3 & 4 | Width of the sedes at 90° to the midpoint between 2 and the left (3) and right (4) edge of the sedes |
| Sagittal (left & right) | 1 | Linear length of the sagittal face beginning and ending at the innermost edge of the annulus |
| | 2 | Distance between inner edges of the annulus |
| | 3 | Maximum height of the glossopodium |
| | 4 | Width of the glossopodium at the midpoint of 3 |
| | 5 & 6 | Width of the glossopodium at the midpoint between 4 and the proximal (5) and distal (6) ends of 3 |

cells are a series of large, lighter staining cells that are predominantly isodiametric in most sections, though they become slightly palisade-like in the dorsiventral sections through the proximal portion of the annulus (Figs. 7d–f; 10c; 13d). In *I. howellii*, these larger, peripheral cells are less obvious, but remain detectable (Figs. 8–10). The outermost layer of the annulus is always formed from these cells (Figs. 5b–c; 7e–f; 8b, e–f; 9b–d; 10e–f; 12c; 13f). This annular ridge is most prominent at the distal end of the cornua (Fig. 2, [adaxial face]), and is generally thinner but still present between the cornua (Fig. 2, [adaxial face]; 5d; 8d; 12d).

Internal to these larger cells is the ground tissue of the glossopodium consisting of cells that are mostly isodiametric in shape, and are not formed into ranks, instead appearing unorganized (Figs. 5–13). These core cells are slightly smaller than the peripheral cells, and decrease gradually in size toward the medimoles. The medimoles stains slightly darker than the other

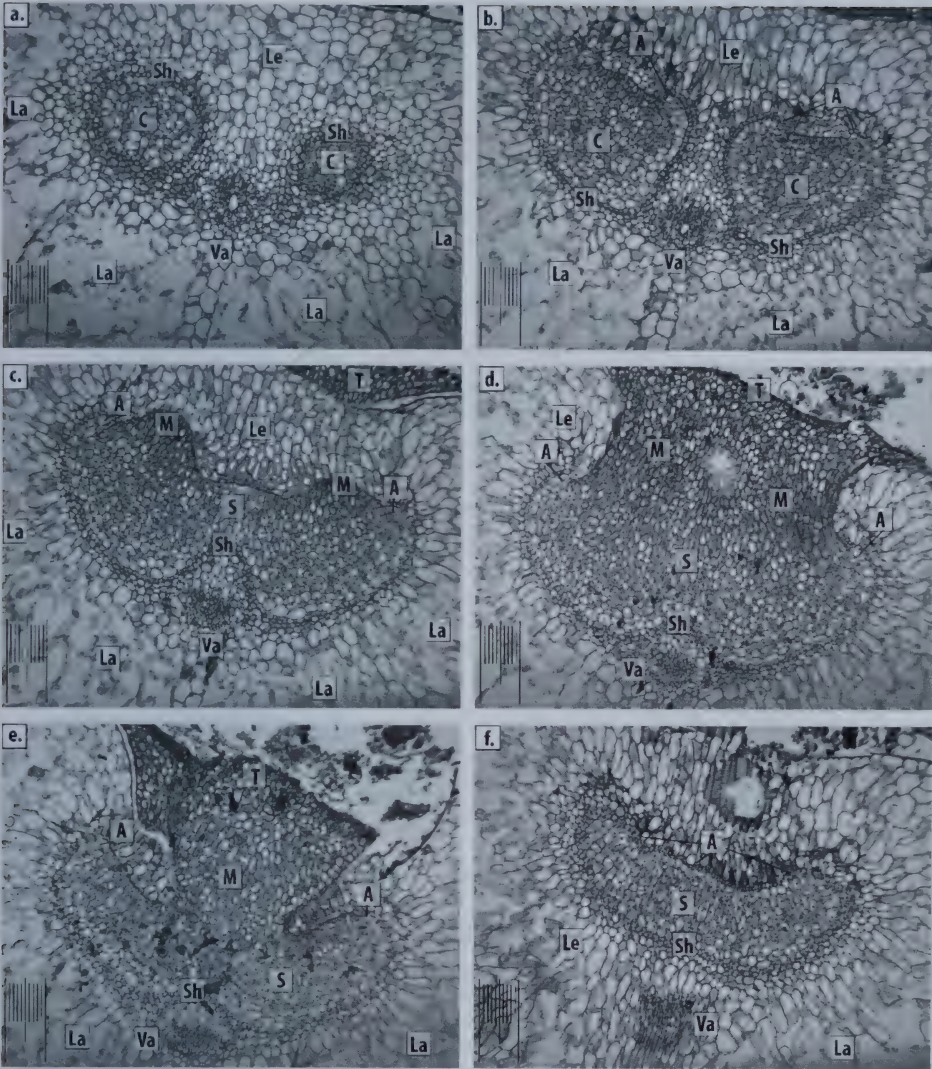


FIG. 5. Transverse sections from *Isoetes bolanderi*, progressing sequentially from most distal (a) to most proximal (f). A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

tissue of the glossopodium, for unknown reasons (Figs. 5b-e; 6b-e; 7d-f; 8b-f; 9b-e; 10c-f; 11b-f; 12c-e; 13d-f), whereas the cells become increasingly elongated parallel to the structure's surface as the medimoles transitions into the tongue (Figs. 6b-f; 8b-f; 12c-f).

In the leaf tissue between the adaxial face of the leaf and glossopodia there are lignified cells with what appear to be helical or reticulate 2° wall thickenings, though the number and density of these cells varies between species. These lignified cells are adjacent to the sheath cells on the adaxial face of the glossopodia, and extend into the surrounding tissue, including the

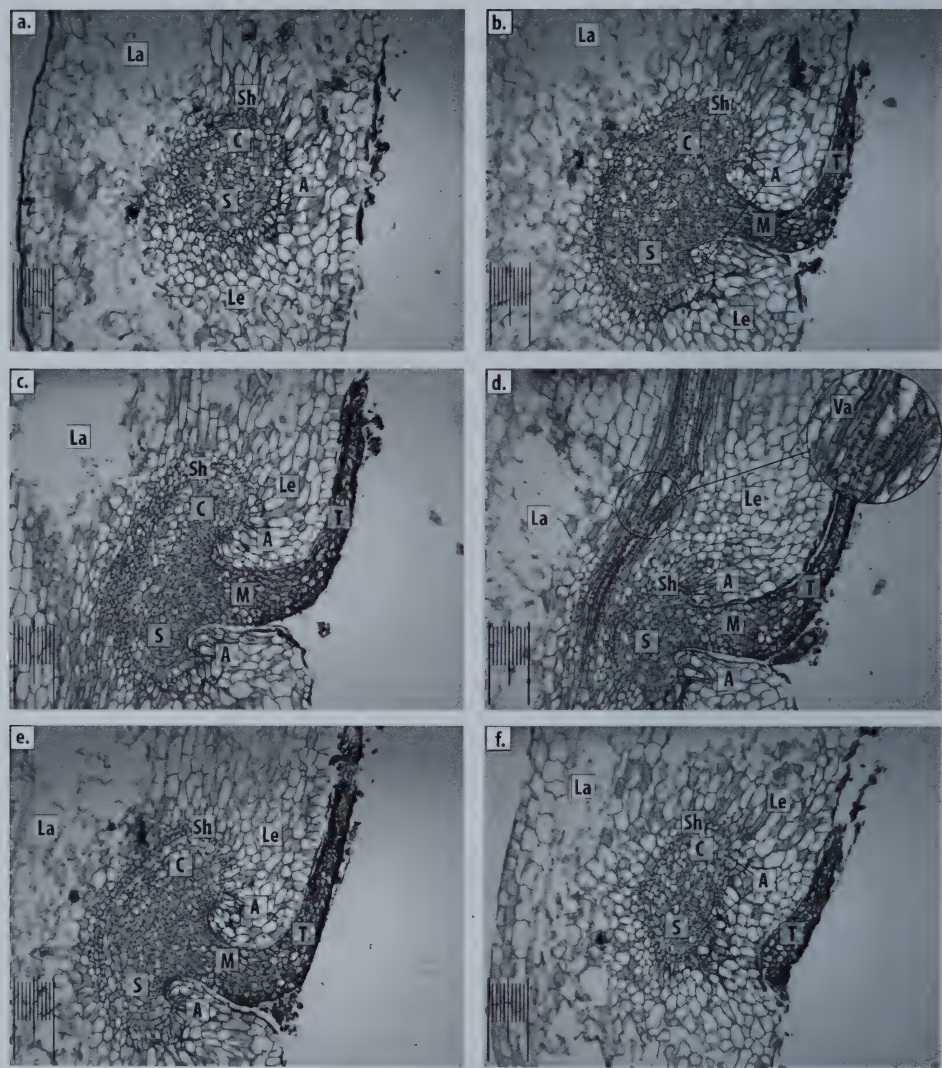


FIG. 6. Sagittal sections from *Isoetes bolanderi*, progressing sequentially from right-most side of the leaf (a) to left-most side (f). A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

labium (Fig. 3). These cells did not connect to the leaf vascular trace in any of the specimens examined.

Three of the leaves sampled possessed a doubled glossopodia, which, if the divided glossopodia were fused together, would form a single unit. For the two samples of *I. nuttallii*, the divided ligule resulted in two partially formed glossopodia (Fig. 14), with both halves having a medimoles and tongue. This differed from the double glossopodia found in *I. bolanderi*, which was a detached cornu with its own medimoles and tongue (Fig. 14).

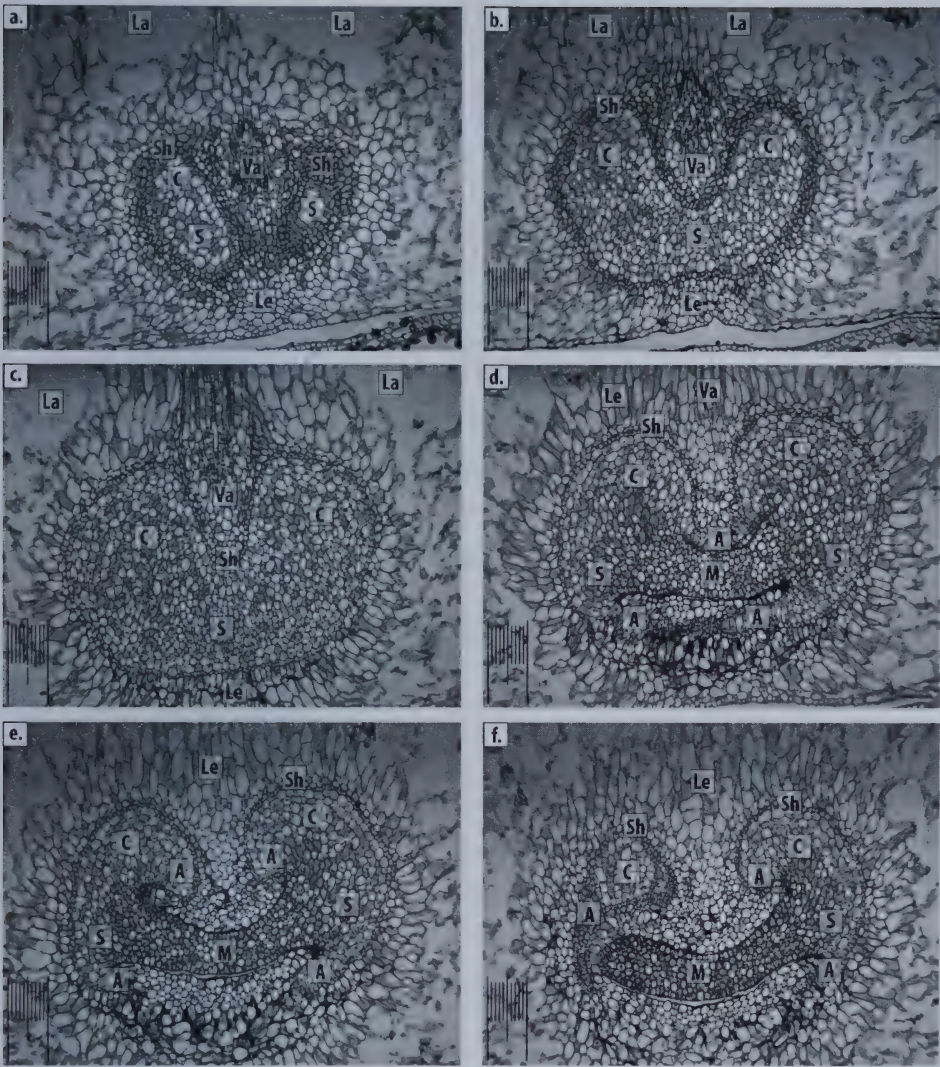


FIG. 7. Dorsiventral sections from *Isoetes bolanderi*, progressing sequentially from most abaxial (a) to most adaxial (f). A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

Anatomy and morphology of the reconstructed glossopodia.— Descriptions of the anatomy and morphology of the glossopodia are organized below by species. The terminology largely follows Shaw and Hickey (2005). Descriptions use the terms “left” and “right” to describe the structures with the orientation of the distal portion upward, and facing the abaxial surface. The glossopodium is considered to have transitioned into the medimoles when the tissue of the glossopodium diverges out towards the leaf adaxial surface inside the annular ridge. General descriptions of the overall shape

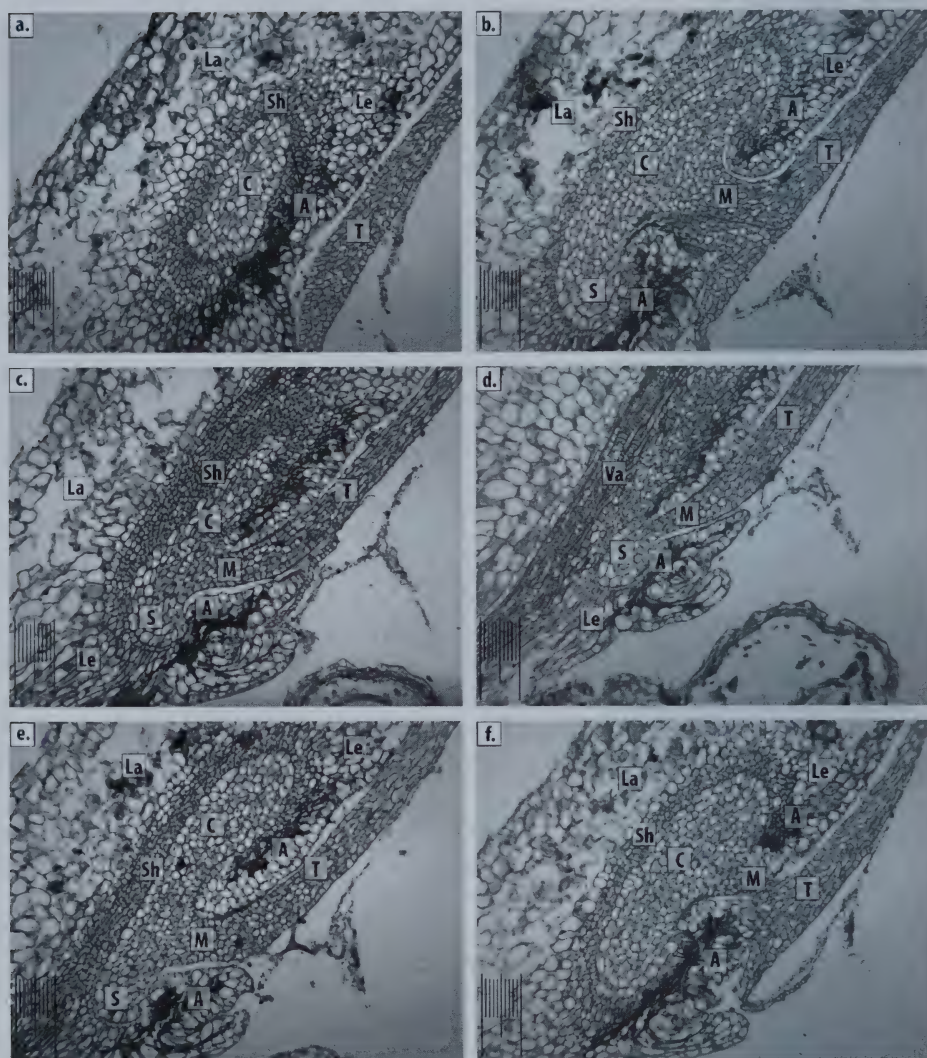


FIG. 8. Sagittal sections from *Isoetes howellii*, progressing sequentially from right-most portion of the leaf (a) to left-most portion (f). A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

and size of the glossopodia can be found in Table 3. In all species examined, the annulus is widest at the apex of the cornua and thinnest at the base of the gap between them (Fig. 2 [adaxial face]).

ISOËTES BOLANDERI.—The glossopodium of *I. bolanderi* is proportionally thicker in sagittal width when compared to the other two species, and has little curvature towards the adaxial or abaxial faces of the leaf (Fig. 2B, Table 3).

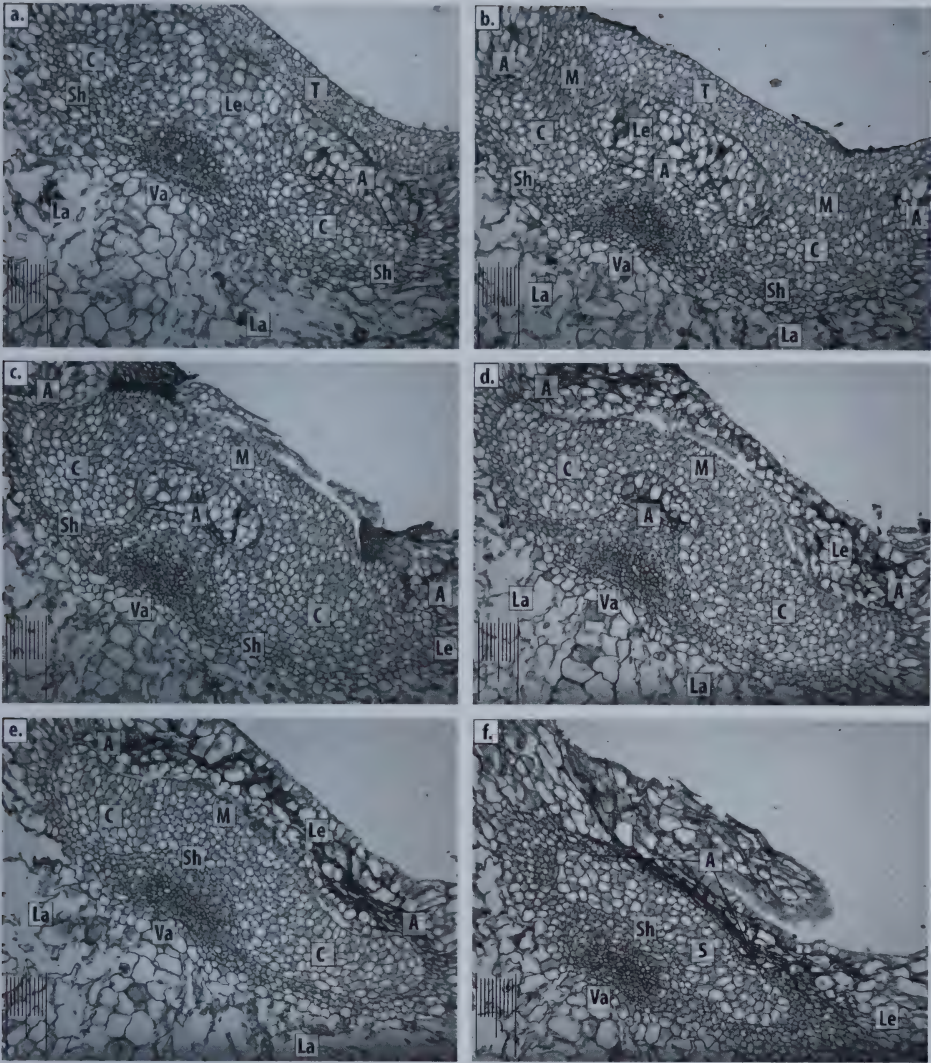


FIG. 9. Transverse sections from *Isoetes howellii*, progressing sequentially from most distal (a) to most proximal (f). A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

The sagittal faces of the cornua are ovoid, with the abaxial face smoothly rounded, and the adaxial side fairly flat, but with a slight hump just below the midpoint (Fig. 2C, D). This hump is visible in the abaxial view, and is widest just above the base of the cornua (Fig. 2F). The cornua account for about 50% of the total length of the glossopodia in the longitudinal axis (Fig. 2A, F). In the distal transverse view, the cornua lobes are quite thick, and slightly triangular to round in shape (Fig. 2B). The glossopodium is slightly indented near where the vascular bundle passes through the adjacent

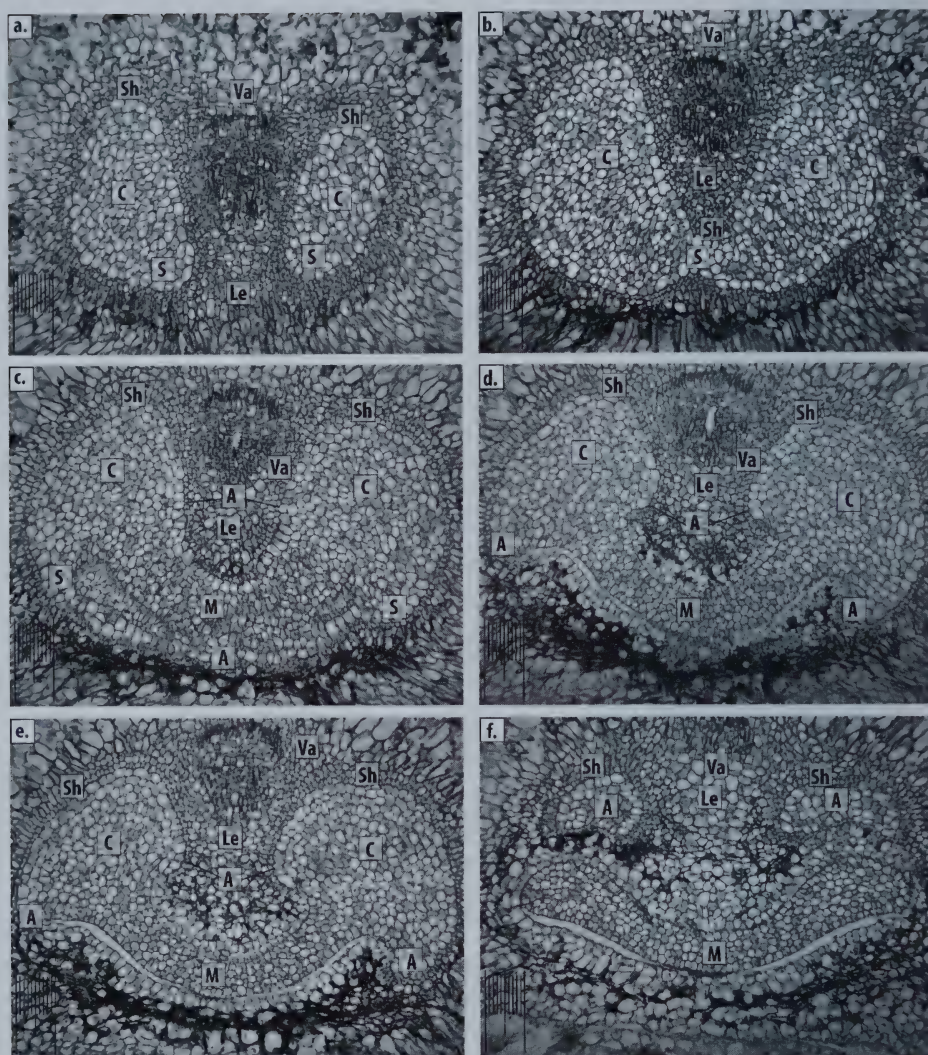


FIG. 10. Dorsiventral sections *Isoetes howellii*, progressing sequentially from most abaxial (a) to most adaxial (f). A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

leaf tissue, but this does not substantially reduce its thickness (Fig. 2B, E). The proximal end of the glossopodium is rounded in both dorsiventral and sagittal views (Fig. 2A, C, D, F).

The medimoles is short and thick, and has a slight upward curvature as it transitions into the tongue. The medimoles follows the curve of the glossopodium, and terminates about mid-way up the cornua (Fig. 2A, 6b-e).

The tracheid-like cells found in the leaf ground tissue are sparse, only appearing in small numbers (Figs. 2a; 5c-d & f; 6b; 7d-f).

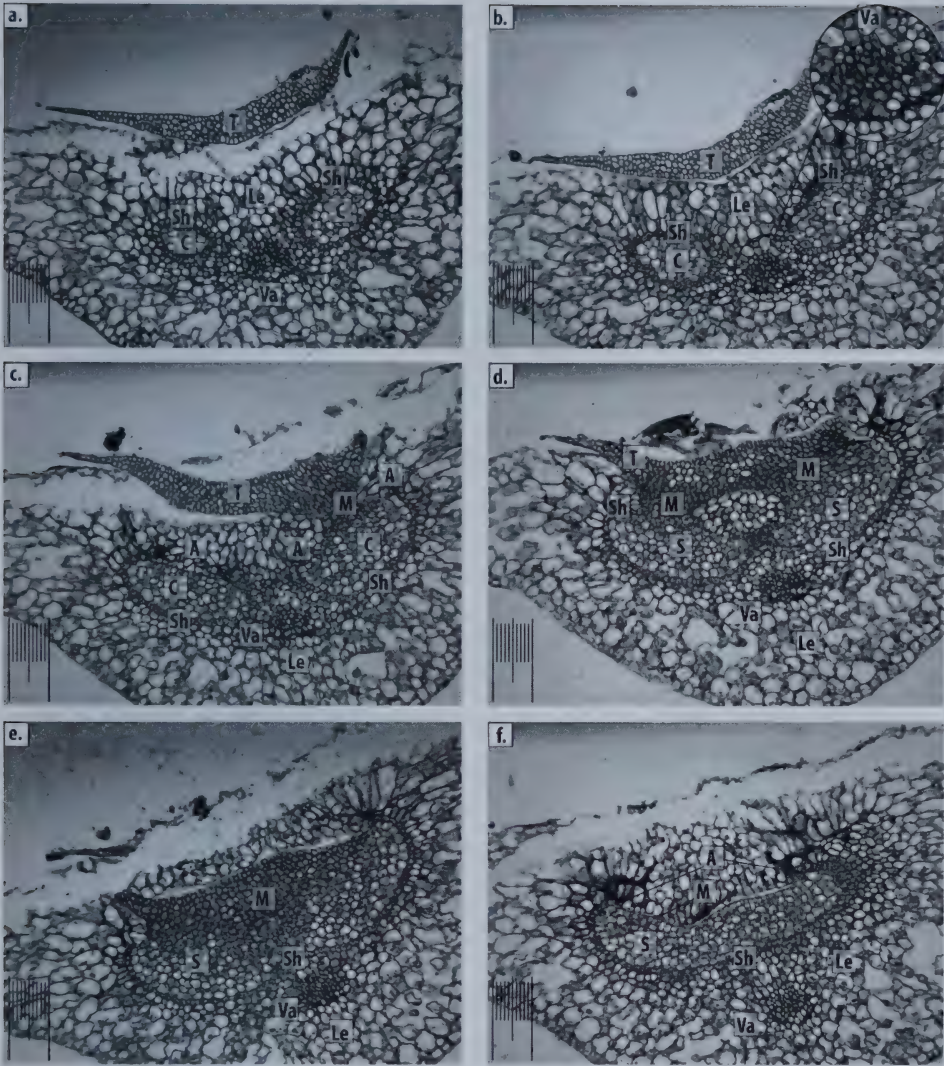


FIG. 11. Transverse sections from *Isoetes nuttallii*, progressing sequentially from most distal (a) to most proximal (f). A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

ISOETES HOWELLII.—The glossopodia of *I. howellii* are larger than those of *I. bolanderi* and are quite different in general appearance (Table 2). Rather than being a thick, stout structure, it is a broad, flattened structure when viewed in the sagittal or transverse plane (Fig. 2H, K). In sagittal view it is proportionally quite thin, and of fairly even thickness for most of its length, although some specimens narrow near the proximal end, with the abaxial side of the glossopodium slightly rounded and the adaxial side more or less flattened (Fig. 2I, J). In abaxial view, the cornua are quite long, becoming broadest near

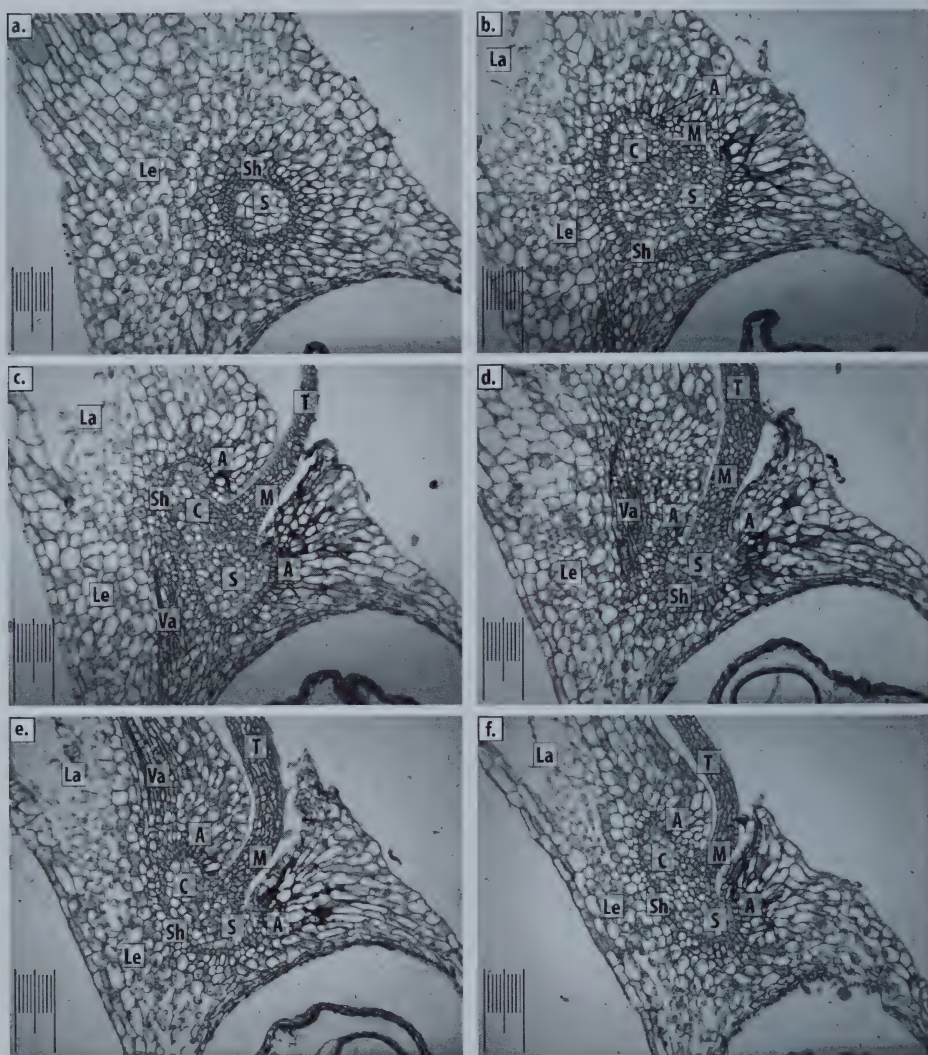


FIG. 12. Sagittal sections from *Isoetes nuttallii*, progressing sequentially from right-most portion of the leaf (a) to left-most portion (f). tissue. A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

the midpoint of the lobes; these structures comprise the majority of the structure's volume (Fig. 2G, L). The indentation on the abaxial face is most noticeable in the distal and proximal view, though it is not very deep (Fig. 2H, K). Like *I. bolanderi*, it has a rather rounded proximal end in sagittal view (Figs. 2I, J; 8).

The medimoles itself runs most of the way up the cornua, yet has a fairly level apex near the ligule, giving it a hollowed out, half-domed shape. The

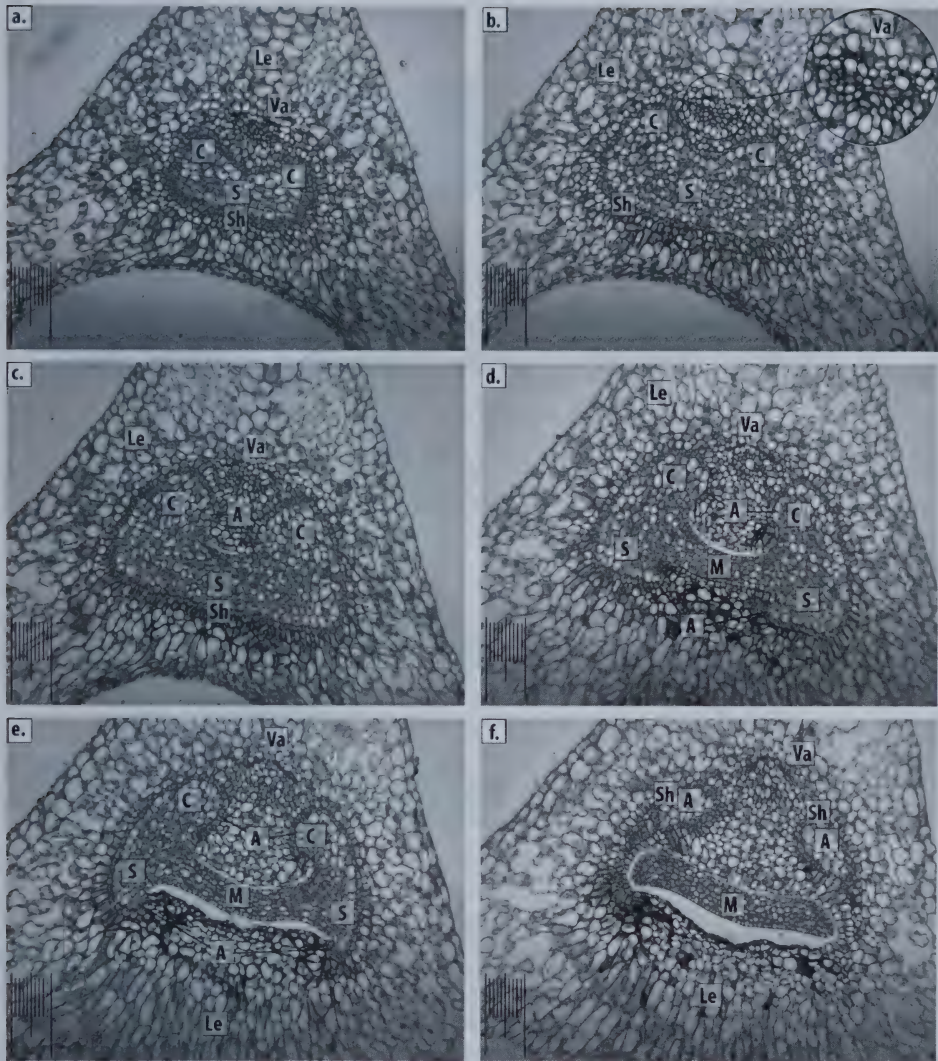


FIG. 13. Dorsiventral sections from *Isoetes nuttallii*, progressing sequentially from most abaxial (a) to most adaxial (f). A) Annulus. C) Cornua. Sh) Sheath cells. La) Lacuna. Le) Leaf tissue. M) Medimoles. S) Sedes. T) Tongue. Va) Vascular tissue. Scale = 0.1mm with 0.01mm increments.

medimoles is also rather short, with the glossopodium near the adaxial surface of the leaf (Figs. 8–10).

The tracheid-like cells are dense in the space between the glossopodium and the adaxial epidermis, and are reticulate in their 2° wall thickenings (Figs. 3b).

ISOËTES NUTTALLII.—The glossopodium of *I. nuttallii* is remarkably different from the preceding two species, and is the smallest in size (Table 3). The greatest

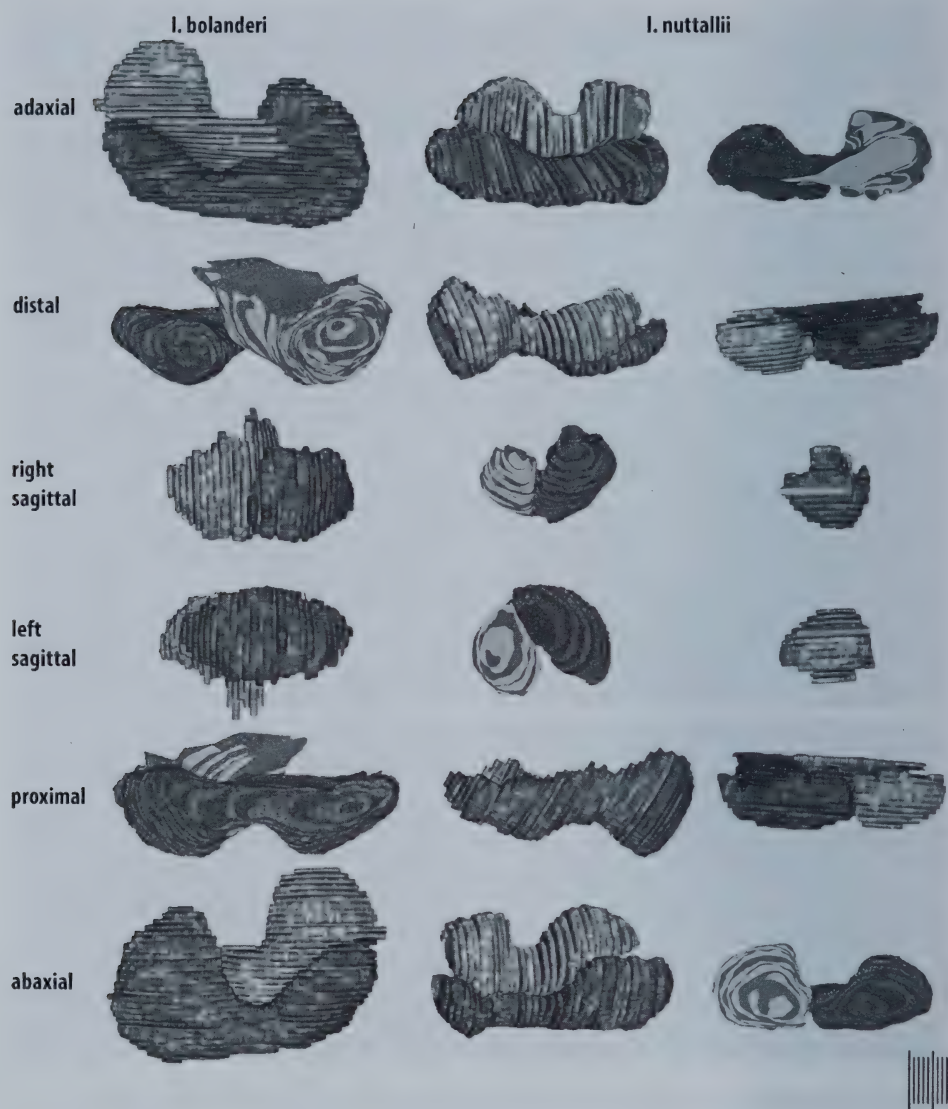


FIG. 14. 3D projections of the divided glossopodia found during the reconstruction process. The twinning is emphasized in the reconstruction by performing a color inversion on one portion of the stack, representing one part of the divided structure. Scale = 0.1mm with 0.01mm increments.

difference between *I. nuttallii* and the other two species is that the glossopodium is bowed inward toward the adaxial face (Fig. 12b–c) giving the structure a triangular and thickened appearance in sagittal view (Fig. 2O, P), even though the absolute thickness of the glossopodium as a structure is actually fairly thin. The distal-most portions of the cornua are acutely pointed in dorsiventral view, and are short compared to the entire height of the

TABLE 3. Comparisons of glossopodial morphology of *Isoetes bolanderi*, *I. howellii* and *I. nuttallii*.

| | <i>I. bolanderi</i> | <i>I. howellii</i> | <i>I. nuttallii</i> |
|--|---|------------------------------------|--------------------------------------|
| Dorsiventral view of cornua | Ovate | Ovate to broadly triangular | Stout triangular |
| Sagittal view of cornua | Reniform | Thin, narrow elliptic | Stout triangular |
| Ligule attachment position on glossopodium | Slightly above center, ascending the cornua | High, ascending the cornua | Slightly above center, non-ascending |
| Medimoles size | Short, well developed | Short, extensive | Long, not extensive |
| Medimoles angular departure from the glossopodium | Ascending | Ascending | Perpendicular |
| Angle of the glossopodium compared to vertical leaf axis | Distal portion leans adaxially | Nearly parallel with the leaf axis | Nearly parallel with the leaf axis |
| Mean cornua height (mm) | 0.5 | 0.8 | 0.3 |
| Mean sedes width (mm) | 0.9 | 1.0 | 0.7 |

glossopodium (Figs. 2M, R). The glossopodium is thickest at the proximal end, giving the whole structure an almost trapezoidal shape in abaxial view (Fig. 2R). The proximal face is rather flattened, and is bowed towards the adaxial face of the leaf in distal or proximal view (Figs. 2M, N, Q, R; 12b–d). The indentation near the vascular bundle is shallow (Fig. 2N, Q).

The medimoles base has the least amount of upward curvature in this species, and is nearly linear in shape in adaxial view (Figs. 2M; 14d–f). In sagittal view, it only curves up slightly as it transitions into the tongue. The medimoles is long proportionally, with the glossopodia seated deeply into the leaf (Fig. 13c–f).

While not as abundant as in *I. howellii*, the tracheid-like cells are present and quite visible in the sections where they occur (Figs. 3c; 11b–f; 12).

Linear Distances.—MANOVA of linear distances demonstrates that the glossopodia of the three species differ significantly from one another in all faces (Table 3). The TukeyHSD post hoc tests indicate that *I. howellii* and *I. nuttallii* differ in 34 of 36 Euclidean measurements, while *I. bolanderi* and *I. nuttallii* have significant differences in 32 of 36 Euclidean measurements; *I. bolanderi* and *I. howellii* showed the greatest similarity, differing in only 13 of the 36 Euclidean measurements (See Appendix 8 on Morphobank for statistics). For all three species, the linear distances that were always significantly different were Abaxial 1–3, 6, and both Left and Right Sagittal 2 and 3, which pertain to the combined length of the sedes and cornua (Fig. 2, Table 4). The only other measurement that had consistently significant differences was Distal 7, which relates to the absolute width of the glossopodium from the distal view.

Comparing *I. bolanderi* and *I. howellii*, the only measurements that showed significant differences were those related to the position of the two cornua to one another (Distal 5, 6, 9). Between *I. bolanderi* and *I. nuttallii*, nearly all of the linear distances are significantly different, with the exception of the width at the base of the sedes (Abaxial 5), and the width of the saddle between the cornua (Distal 6 and 9). This contrasts with the comparison between *I. howellii* and *I. nuttallii*, where the one area with no statistically significant differences relates to the midpoint of the sedes (Abaxial 7 and Proximal 2).

Elliptical Fourier Analysis [EFA].—EFA reveals that there are differences in the shape of the glossopodia among the three species. Of the different projections analyzed, the two faces with the tightest intraspecific clustering in PCA analysis are the abaxial face (Fig. 15a), and the medimoles base (Fig. 15b). In the latter, all three species formed exclusive clusters with no overlap in either the projection's points or the 95% confidence limits around the centroids (Fig. 15b). For the former, *Isoëtes howellii* was the most distinctive, whereas *I. bolanderi* and *I. nuttallii* were slightly more similar to one another in shape. Though all three species did have discrete 95% confidence limits, two of the *I. nuttallii* specimens were within the 95% confidence interval of *I. bolanderi*, while the reverse was not true (Fig. 15a).

Among the other projections, there was substantially less discrimination among the shapes of different species. In the left and right cornua, there was considerable overlap among samples and within the 95% confidence limit and

TABLE 4. Multivariate analysis of variance (MANOVA) of inter-landmark distances from five projections (faces) of the 3D reconstructions of the glossopodia of *Isoëtes bolanderi*, *I. howellii*, and *I. nuttallii*. Results reflect log-transformed data, evaluated using Pillai’s trace. DF = Degrees of freedom. Num DF = Degrees of freedom of the dependent variables. Den DF = degrees of freedom of the model.

| Face | DF | Pillai | Approximate F | Num DF | Den DF | p-value | Residuals DF |
|--------------|----|--------|---------------|--------|--------|-------------------------|--------------|
| Abaxial | 2 | 1.5393 | 3.0377 | 22 | 20 | 0.007627 | 19 |
| Distal | 2 | 1.5884 | 5.1456 | 18 | 24 | 0.0001361 | 19 |
| Proximal | 2 | 1.4481 | 11.1500 | 8 | 34 | 1.441x10 ⁻⁰⁷ | 19 |
| Left cornua | 2 | 1.7296 | 15.993 | 12 | 30 | 7.253x10 ⁻¹⁰ | 19 |
| Right cornua | 2 | 1.6985 | 14.083 | 12 | 30 | 3.42x10 ⁻⁰⁹ | 19 |

the shape of *I. nuttallii* and *I. bolanderi*, although these were both quite distinct from *I. howellii* (Fig. 15c–d).

The distal and proximal faces were quite distinctive overall among species, but still showed some overlap of samples and 95% confidence limits suggest higher intraspecific variance in these projections (Fig. 15e–f).

Of note is that almost all of the variability in the data was explained by the first eigenvector of the abaxial face, medimoles, and cornua lobes (Fig. 15a–d). By contrast the distal and proximal faces also showed high loadings on the first eigenvector and there was also considerable variance in shape accounted for by the second (Fig. 15e–f).

DISCUSSION

This study adds a statistical, morphometric approach to the more traditional qualitative one used by previous authors (Sharma and Singh, 1984; Shaw and Hickey, 2005). The goals were to develop a repeatable technique for characterizing the shape and size of the glossopodia and to determine whether the three sampled species differ in glossopodial features. Even with the limited sampling in this study, both MANOVA (Table 3) and EFA (Fig. 15) reveal significant differences in both the shape and size of the sampled glossopodia, which are augmented by the qualitative differences observed in the 3D-reconstructions (Fig. 2). Others can follow these techniques and analyses and place this quantitative variation in a comparative, phylogenetic context. Ultimately, glossopodial traits can be evaluated for functional or evolutionary correlations, and used as a taxonomic character to distinguish species.

Comparing the glossopodial reconstructions in this study to those of Shaw and Hickey (2005), a few trends bear mentioning. Although it was not possible to develop quantitative comparisons between the species analyzed here and those included in their work (*I. melanopoda*, *I. virginica*, and *I. tennesseensis*), qualitatively their reconstructions are all fairly similar in general appearance to the reconstructions of *I. howellii* and *I. bolanderi*. This similarity is encouraging because these five species are members of the same widespread North American clade of *Isoëtes* (Hoot *et al.* 2006), suggesting that related



FIG. 15. Principal component factor loading plots from the elliptical Fourier analysis of the glossopodia outlines, using six different projections (a–f). Triangles represent the individuals sampled in the study. Ellipses represent the 95% confidence limit for the centroid of each species. The bar graph in the lower right-hand corner of each panel shows the loadings for each principal component. X and Y axes are labeled with their corresponding principal component, and their loading percentage. B) *Isoetes bolanderi* (red). H) *I. howellii* (green). N) *I. nuttallii* (blue). From left to right, top to bottom: a) Abaxial face. b) Medimoles base. c) Left cornua lobe. d) Right cornua lobe. e) Distal face. f) Proximal face.

species may share consistent glossopodial morphological qualities. Conversely, it is encouraging that *I. nuttallii* is different both quantitatively and qualitatively from the other species examined. While it is premature to make any strong statements about the overall usefulness of glossopodia as a tool to discriminate between lineages at this time, the presence of these differences

are encouraging, as they indicate the value of continuing to examine the glossopodium in a morphometric context. With additional sampling, it might be possible to determine if the glossopodia's shape has strong phylogenetic signal (as these results suggest), or if it is associated with another trait, such as leaf gender, location in the rosette, ploidy, or the plant's habitat.

Along with an increased sample size, it will be pertinent to determine how the size and shape of the glossopodia vary based on leaf size. The leaves of *Isoetes* show significant intraspecific variation in length and width, likely related to their environmental conditions (Freund, personal observation; Kott and Britton, 1985; Pfeiffer, 1922). If the size of the glossopodium varies in proportion to the leaf, then shape becomes much more valuable than absolute size.

The divided ligules found in three of the specimens, which have not been previously documented, raises some developmental questions that are beyond the scope of this study. First, it is not clear how these twinning events occurred, but given that the structures begin as a single differentiated cell on the epidermal layer of the young leaf and then rapidly achieve their final form as the leaf matures (Bhamie *et al.*, 1963; Sharma and Singh, 1984; Smith, 1900), it is possible that the division of the glossopodium occurred early in development. The divided structures each possessed a tongue, medimoles, and glossopodia, the latter of which are incomplete individually, but together form a complete unit. This construction suggests that the developmental series of the glossopodium itself may be deterministic. While developmentally interesting, these anomalies indicate that any study of glossopodia should sample multiple leaves per individual in order to understand intra-individual variability, as well as multiple individuals per population. Such sampling would help to determine the frequency of these aberrant ligules in a given species or population, and may help improve our understanding of the changes in the developmental pathway of the leaf during the diversification of *Isoetes*.

Nonetheless, there are issues with applying this methodology in broad-scale comparative studies. One is the time investment required. Glossopodia are too soft to be easily dissected from the leaf, and cannot be readily examined in the field. Additionally, they must be sectioned from fresh or pickled material, as the leaves shrink and distort considerably when dried as herbarium specimens. In addition there is the issue of producing a sufficient number of sections to resolve a 3D object that accurately represents the shape of the glossopodium. The technique described here works well on larger glossopodia, but for smaller glossopodia, such as those of *I. nuttallii*, sections need to be taken at intervals of 5µm or less to adequately characterize shape. This may limit analysis of exceptionally small glossopodia. By applying Micro-CT (M-CT) scanning (Bogart *et al.*, 2010; McElrone *et al.*, 2013), it may be possible to produce a viable reconstruction of a glossopodium in its entirety, eliminating the need for histological sectioning. This approach would yield a higher resolution image for interpretation, as the M-CT sections are far thinner than those that can be acquired using a microtome. Additionally, because the reconstructions are obtained as the scan is performed, the potential for errors

in aligning the glossopodial slices is reduced, resulting in a clearer interpretation of the true shape of the structure. And finally, whereas histological sectioning can take weeks to make a set of reconstructed glossopodia from a few leaves, M-CT can potentially scan a full rosette in about an hour. This approach would sample more glossopodia and would identify the placement of the leaves being interpreted on the rosette, the gender of the leaves, and even their stage of development. Given these potential benefits, this method should be seriously considered in the future.

The technique described in this paper is important for studying the morphology of *Isoëtes* because it is statistically robust and reproducible. Most prior studies of glossopodia have lacked these characteristics. Consider, for example, two prior studies of the glossopodia in *I. coromandelina* from the Indian subcontinent. The first of these (Sharma and Singh, 1984) was one of the primary sources of information on the morphology of the glossopodia (Gifford and Foster, 1989; Shaw and Hickey, 2005). However, an earlier study (Bhambie, 1963), also of the glossopodia of *I. coromandelina*, described radically different shapes. Sharma and Singh (1984) reported two pairs of cornua lobes, one facing distally, one proximally, together resulting in an “H” shaped structure. By contrast, Bhambie (1963) interpreted the structure as having only a single pair of thick cornua that more closely resemble those described here and by Shaw and Hickey (2005). However, given that neither Sharma and Singh nor Bhambie explained how the structures were reconstructed, interpreted, and illustrated, it is impossible to determine if one of the renditions is more accurate, or whether both descriptions are accurate but are from different cryptic species (perhaps of different ploidy levels) within the *I. coromandelina* complex (Jung *et al.*, 2014).

Clearly, there is a need for improving our understanding of both the taxonomy and phylogeny of *Isoëtes* to provide a framework for analyses of morphological variation. The technique applied in this study yields results that demonstrate significant differences among the glossopodia that can potentially be species specific. Use of methodologies that are reproducible and statistically sound is vitally important if our conclusions are to have weight. And, as all but one of the programs used in the analysis are free-ware, this methodology does not have a major software cost to limit its use.

1476 × 1530

Conclusions.—This study provides the first multivariate morphometric analysis of glossopodia size and shape, showing that the glossopodia of *Isoëtes* differ significantly among three California species. This approach could be particularly helpful in evaluating evolutionary change of the glossopodia within a phylogenetic context, which will be important as we develop an understanding of the function(s) of this enigmatic structure. Although it is unlikely that this structure alone could be taxonomically discriminatory for all species, it has potential utility in combination with the suite of characters generally used in the identification and description of *Isoëtes* species (e.g., megaspore morphology, vellum features, etc.).

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The Hybrid between *Equisetum scirpoides* and *E. variegatum* in Northern Europe

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ABSTRACT.—Based on newly collected plant material from Swedish Lapland a new name, *Equisetum* \times *lapponicum*, is proposed for the hybrid between *E. scirpoides* and *E. variegatum*. Macromorphological as well as micromorphological characters are presented in comparison with the parent species. The nomenclatural history of the hybrid combination is discussed in detail referring to older but invalid names used in the literature. An updated overview of reticulate evolutionary processes in subgenus *Hippochaete* at a global scale is presented.

KEY WORDS.—Equisetaceae, taxonomy, micromorphology, diagnostic characters, reticulate evolution

Horsetails are an ancient group of spore producing vascular plants, currently represented by only a single genus *Equisetum* comprising 15 species worldwide (Hauke, 1963, 1978). The genus is commonly divided into two subgenera, *Equisetum* and *Hippochaete*, which distinctly differ on the sporophytic as well as on the gametophytic level, so that two separate horsetail genera could be raised (Milde, 1865b). Hybridization is a common phenomenon within (but never between) both subgenera of the Equisetaceae; 19 diploid hybrids have been described and published up to the present (see synopsis in Lubienksi, 2010; plus Page and Gureyeva, 2009). In subgenus *Hippochaete* evolutionary processes seem to be more complex than previously presumed, which is attested by three triploid hybrids, one of which incorporates three different genomes, detected in central Europe in the last decade (Bennert *et al.*, 2005; Lubienksi and Bennert, 2006; Lubienksi *et al.*, 2010). *Equisetum scirpoides* Michaux remained to be the only species within subgenus *Hippochaete* not involved in any hybrid combination (following Hauke [1963] in his monograph and treating *E. debile* Roxb. ex Vauch. from Southeastern Asia as a subspecies of *E. ramosissimum* Desf.). Also, Duckett (1979) failed to synthesize hybrid offspring involving this species in his experimental hybridization studies. Plants of supposed hybrid origin between *E. scirpoides* and *E. variegatum* Schleich. ex F. Web. & D. Mohr on the other hand have long been presumed, discussed or rejected in the botanical history and were repeatedly mentioned in literature (Holmberg, 1920;

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Hiitonen, 1933; Rothmaler, 1951; Hylander, 1953; Almquist and Björkman, 1960; Hauke, 1963; Borg, 1967; Meusel *et al.*, 1971; Cody and Britton, 1989; Mascher, 1990; Hauke, 1993; Øllgaard and Tind, 1993; Øllgaard, 2000). However they neither have been confirmed nor have any names for them been validly published. Here we describe the hybrid based on recently found material from Swedish Lapland and propose a new name for it.

MATERIAL AND METHODS

Plant material used in this study is presented under additional specimens examined. In every case small samples were taken into cultivation by rhizome cuttings to enable long term observation under controlled conditions. Voucher specimens including the isotype of *E. ×lapponicum* were additionally deposited at the public herbarium of the LWL-Museum für Naturkunde in Münster, Germany (MSTR), with the exception of the holotype, which is at the Swedish Museum of Natural History in Stockholm, Sweden (S).

Spore material was obtained from pressed and air-dried fertile shoots by opening the sporangia and embedding the spores in Corbit-Balsam. The slide preparations were dried for 24 hours.

Microphotography was done with a digital microscope (Keyence VHX 500F) equipped with a high-precision VH mounting stand with X-Y stage and bright field illumination (Keyence VH-S5).

For scanning electron microscopy (SEM) studies, freshly collected shoots were fixed for 72 h in FAA (100 ml FAA = 90 ml 70% ethanol + 5 ml acetic acid 100% + 5 ml formaldehyde solution 37%) before being stored in 70% ethanol for 48 h. For SEM with AURIGA ZEISS TM the fixed material was dehydrated in FDA for 24 h (formaldehyde-dimethyl-acetal) and critical-point dried (CPD 030, BALZERS). CP-dried material was sputtered with gold-palladium (thickness 5 nm) with a BALTEC Sputter-Coater SCD 030.

RESULTS

Equisetum ×lapponicum Lubienksi & Dörken, **hybr. nov.**

(*E. scirpoides* Michx. \times *E. variegatum* Schleich. ex F. Weber & D. Mohr)

TYPE. — SWEDEN. Lycksele Lappmark: Artfjället, Gausjosjön, near Strima-sund, wet marsh on the eastern shore of Över-Uman, 26 km north of Hemavan, Storums kommun, 66°03'06.2"N /14°51'25.9"E, 530 m a. s. l., 16 Jul 2014, M. Lubienksi 1166 (holotype: S!; isotype MSTR 164872!) **Figs. 1; 2 A–D; 3 B; 4 B; 5 B; 6; 7 B**

Diagnosis and description.—*Planta hybrida; parvae herbae altitudinis habitusque inter Equisetum scirpoides et Equisetum variegatum; condicionibus incrementi auctis (exempli causa in cultura) Equiseto variegato magis*

atque magis aequantes; caules tenues, accumbentem usque ad erectum habitum ostendentes; congregati; caulis nudus (interdum unum ramum lateralem proxime sub strobilo imminuto proferens); sempervirentes, 7-15 cm altitudinis et 1-3 mm diametri, cum fere 4-5 (vel raro 6) carinis lineas duas tuberculorum separatorum proferentibus; valleculae cum rosulis sulcisque duobus separatis stomatorum; lacuna centralis caulibus minimis deest, maioribus autem inest; vaginae, continue nigricant vel marginem nigrum ostendentes virent; dentes nigri, oblongi, cum arista atra magisque minusve persistenti et cum fere angusto, subalbo margine cutis; caulibus teneris crescentibus arista a caule distenditur similiter Equiseti scirpoidis; strobilus parvus, angustus, imminutus; sporangiae se non aperiunt; spores subalvae, non constanter formatae, sine elateribus.

Plants small, in size and growth habit intermediate between *Equisetum scirpoides* and *E. variegatum*, becoming increasingly similar to *E. variegatum* under more favorable conditions (e.g. in cultivation), shoots thin, decumbent to upright, clustered, unbranched (sometimes with a single side branch just beneath the stunted strobilus), evergreen, height 7–15 cm, diameter 1–3 mm, with usually 4–5 (rarely 6) ridges, these with two rows of distinct silica tubercles, furrows with rosettes of silica and two distinct rows of stomata, central cavity absent in very small shoots and present in larger ones, sheaths blackish throughout or green with a black rim, sheath teeth black, oblong, with a dark \pm persistent awn and a mostly narrow whitish scarious margin, the awn spreading from the shoot in young emergent shoots similar to *E. scirpoides*, strobili small, slender, and stunted, with 4–5 whorls of sporophylls, sporangia not opening, spores whitish, achlorophyllous, irregular formed, lacking elaters.

Additional specimens examined.—NORWAY. **Nordland:** Fiplingdalen, Børgfjellet, wet depression near Nedre Fiplingvatnet, 04 Aug 2001, *M. Lubienski ML 68* (MSTR 164873!). SWEDEN. **Torne Lappmark:** east of Abisko, Stordalens naturreservat, wet area south of Torneträsk lake, 1987, *U. Peters ML 39* (MSTR 164871!).

E. scirpoides.—CANADA. **Alberta:** Jasper National Park, Hot Springs, 2008, *W. Jäger ML 225* (MSTR 164842). FINLAND. **Koillismaa:** Salla, Oulangan kanjoni, Oulankajoki, 19 Jul 2012, *M. Lubienski ML 292* (MSTR 164845). NORWAY. **Oppland:** Gudbrandsdalen, Tromsa valley, near Brekkom, 01 Aug 2001, *M. Lubienski ML 66* (MSTR 164844). SWEDEN. **Torne Lappmark:** Abisko, 1962, cultivated *Bot. Garden Tromsø ML 41* (MSTR 164842). **Lycksele Lappmark:** Artfjället, Gausjosjön, near Strimasund, marsh near the eastern shore of Över-Uman, north of Hemavan, 16 Jul 2014, *M. Lubienski ML 308* (MSTR 164843).

E. variegatum subsp. *variegatum.*—AUSTRIA. **Tirol:** west of Kufstein, at the Glemmbach near Landl, 01 Oct 2008, *M. Lubienski ML 223* (MSTR 164850). BELGIUM. **Luxembourg:** Etalle, northeast of Huombois, old quarry near Fourneau Marchand, 31 Oct 2010, *M. Lubienski ML 263* (MSTR 164853). ESTONIA. **Lääne Maakond:** Nooarotsi poolsaar, gravel pit northwest of Hara, 30 Jul 2004, *M. Lubienski ML 153* (MSTR 164861). FRANCE. **Bas-Rhin:** near

Fort-Louis, 1955-1960, *F. Geissert ML 128* (MSTR 164854). **Haute-Garonne:** Bagnères-de-Luchon, Val d'Astau, north of Granges d'Astau, rivulet south of Oô, 25 Jul 2005, *M. Lubienski ML 174* (MSTR 164855). GERMANY. **Baden-Württemberg:** small pond near Leutesheim, 18 Feb 2007, *M. Lubienski ML 195* (MSTR 164847). **Baden-Württemberg:** Bad Waldsee, old gravel pit near Schwende, 03 Apr 2013, *M. Lubienski ML 300* (MSTR 164848). **Bayern:** near Bad Reichenhall, 1993, *H.W. Bennert ML 36* (MSTR 164849). **Thüringen:** betw. Meuselwitz and Zechau, old open-cast mine near Altpoderschau, 03 Jul 1999, *M. Lubienski ML 37* (MSTR 164850). GREAT BRITAIN. **Cumbria:** Asby Scar National Nature Reserve, Potts Valley, 2012, *P. Jepson ML 299* (MSTR 164856). IRELAND. **Dublin:** Kilrock, Howth, 2012, *D. Doogue ML 281* (MSTR 164857). **Kildare:** River Barrow, Tankardstown Marsh, 2012, *D. Doogue ML 282* (MSTR 164858). **Kildare:** River Barrow, Grand Canal, Monasterevin, 2012, *D. Doogue ML 284* (MSTR 164860). **Westmeath:** Lough Owel, Royal Canal exit to Mullingar, 2012, *D. Doogue ML 283* (MSTR 164859). NETHERLANDS. **Gelderland:** Doetinchem, Wittenbrinksche Beek, bog north of Hummelo, 13 Oct 2005, *M. Lubienski ML 181* (MSTR 164852). NORWAY. **Nordland:** Hattfjelldal, on wet rock at Hatten mountain, 05 Aug 2001, *M. Lubienski ML 69* (MSTR 164866). **Nordland:** Saltfjellet, Lønsdalen, wet scrubby vegetation near Polar Circle Center, 20 Jul 2009, *M. Lubienski ML 242* (MSTR 164867). **Nordland:** Austerdalsisen, rocky pond above Svartisvatnet, beneath Svartisen glacier, 21 Jul 2009, *M. Lubienski ML 243* (MSTR 164868). **Oppland:** Gudbrandsdalen, Vinstra gorge, west of Vinstra, 31 Jul 2001, *M. Lubienski ML 63* (MSTR 164864). **Oppland:** Gudbrandsdalen, Tromsa valley, near Brekkom, 01 Aug 2001, *M. Lubienski ML 67* (MSTR 164865). SWEDEN. **Lycksele Lappmark:** Atofjället, marsh at Atoklimpen mountain, 14 Jul 2014, *M. Lubienski ML 309* (MSTR 164862). **Lycksele Lappmark:** Atofjället, marsh at Atoklimpen mountain, very small variant, 14 Jul 2014, *M. Lubienski ML 310* (MSTR 164863).

E. variegatum subsp. *alaskanum* (A.A. Eaton) Hultén.—CANADA. **Alberta:** Banff National Park, *M. Matus ML 40* (MSTR 164869). USA. **Alaska:** Prince of Wales Island, *B. Hoshizaki ML 232* (MSTR 164870).

Etymology.—The hybrid is named after its distribution in the ancestral homeland of indigenous Sami people (Lapland, Sápmi), which stretches all the way from central Norway and Sweden across the far north of Finland and into the Kola Peninsula of Russia.

Distribution and ecology.—*E. ×lapponicum* is known from wet depressions and marshes on the eastern shore of Över-Uman, 26 km north of the village of Hemavan and approx. 530 metres above sea level. The lake is situated at the northern fringe of the Artfjället Mountains and is part of the Umeälven River, which rises from the Scandes flowing into the Gulf of Bothnia (Baltic Sea). The whole area is part of the northern boreal zone and is characterized by an annual specific run-off (precipitation minus real evapotranspiration) of 700 – 1000 mm (Sjörs *et al.*, 2004).

Taxonomic notes.—Rothmaler (Feddes Rep. 54: 81, 1944) created the name *×Hippochaete arcticum* Rothm., but without giving a diagnosis or

description, which renders it invalidly published. Additionally, no specimens ascribed to the hybrid between *E. scirpoides* and *E. variegatum* could be found in Rothmaler's collections at JE. Furthermore, the name *E. arcticum* had earlier been established at species rank for plants later subsumed as a variety of *E. arvense* (Milde 1865a). Nonetheless, the name is later repeatedly taken up as *Equisetum* \times *arcticum* Rothm. (*Equisetum arcticum* (Rothmaler) Hylander, Nord. Kärleväxtfl. 1: 14 [1953]) in the literature (Hylander, 1953; Almquist and Björkman, 1960; Hauke, 1963; Borg, 1967; Meusel *et al.*, 1971; Cody and Britton, 1989; Hauke, 1993; Øllgaard and Tind, 1993; Øllgaard, 2000).

A second name, *Equisetum* \times *gamsii* Janchen (*Equisetum Gamsii* Janchen, Catal. Fl. Austr. 1:64 [1956]) has occasionally been used in central Europe based on Janchen (1956) and Dostál (1984) for misidentified material from the Austrian Alps. However contrary to some older records there is no doubt, that *E. scirpoides* was never part of the central European alpine flora and thus could not be a parent of a putative hybrid there. This name also lacks a description and a type collection. We therefore propose a new name.

Identification.—The characters of *E.* \times *lapponicum* and its parent species *E. scirpoides* and *E. variegatum* are shown in Table 1. A general problem rising in this context is the similarity between both parent species in many macro- and micromorphological aspects and the lack of true diagnostic differences. Characters of the dwarf *E. scirpoides* like the long, black awned, and persistent sheath teeth, the characteristic wavy curved growth habit of the shoots, the exclusive 3-ribbed shoot, and the absence of a central hollow cylinder, which usually make the determination of the species unproblematic, seem to merge in *E.* \times *lapponicum* with the high degree of morphological plasticity of *E. variegatum*. This is amplified by the existence of several fixed and stabilized variants in *E. variegatum* (pers. obs.), some of which are very small and equalling the dimensions of *E. scirpoides*. Only the sheath teeth character (length and persistence) is preserved in the hybrid to a certain degree (Figs. 1–4). This character is not present even in the smallest 4-ribbed variants of *E. variegatum*. Therefore spore abortion becomes a valuable feature to identify *E.* \times *lapponicum* (Fig. 5). It is maintained under controlled conditions in cultivation. Additionally very few greenish and spherical spores (Fig. 6) could be observed in our Norwegian and Swedish material. These spores are similar to those we have found in other European diploid hybrids within subgenus *Hippochaete*, e. g. *E.* \times *moorei* Newm. (*E. hyemale* L. \times *E. ramosissimum*) and *E.* \times *meridionale* (Milde) Chiovenda (*E. ramosissimum* \times *E. variegatum*). According to Krahulec *et al.* (1996) we interpret these as diplospores.

The micromorphology of *E.* \times *lapponicum* resembles that of the parent species. All three taxa have ridges with two rows of distinct silica tubercles and furrows with rosettes of silica and two distinct rows of stomata (Table 1, Figs. 7 A–D). In *E. variegatum* obviously two types of ridge micromorphology occur: one with distinct silica tubercles on the ridge surface (Fig. 7 C) and another one lacking any relief structures along the ridge (Fig. 7 D).

TABLE 1. Diagnostic characters of *E. ×lapponicum* and its parent species *E. scirpoides* and *E. variegatum*.

| | <i>E. scirpoides</i> | <i>E. ×lapponicum</i> | <i>E. variegatum</i> |
|---|--|---|--|
| Size | (5-) 7 - 12 (-15) cm | 7 - 15 (-30) cm | (5-) 10 - 40 (-70) cm |
| Main shoot | decumbent to upright, characteristically wavy curved
clustered, unbranched | decumbent to upright
clustered, unbranched, sometimes a single side branch beneath or from within the stunted strobilus
1 - 3 mm
absent (small shoots) or present | upright, sometimes decumbent
clustered, unbranched, sometimes a single side branch beneath the strobilus, when spores are shed
(1-) 2 - 3 (-5) mm
present (sometimes absent in small shoots) |
| Diameter (main shoot) | 0.5 - 1 mm | | |
| Central canal (main shoot) | absent | | |
| nodal sheath teeth (main shoot) | 3
oblong, black, obtuse, with a thin white scarious margin mainly at the base of the tooth
awn black, persistent and spreading from the shoot, shed only in old shoots | 4 - 5 (-6)
oblong, black, acute, with a ± thin white scarious margin
awn black, ± persistent and spreading from the shoot in young and emergent shoots, shed later in the season
ridges slightly narrower than the furrows, sometimes nearly as wide as the furrow | (4-) 5 - 9 (-12)
oblong, black, acute, with a broad white scarious margin
awn black to brown (sometimes whitish), mostly deciduous, never spreading from the shoot
ridges narrower than the furrows, only in the smallest shoots nearly as wide as the furrow |
| width ratio between ridge and furrow (main shoot) | ridges and furrows mostly of the same width (stem therefore pseudo-6-ribbed) | | |
| Micromorphology of main shoot internodes | ridges with two rows of distinct silica tubercles
furrows with rosettes of silica and two distinct rows of stomata | ridges with two rows of distinct silica tubercles
furrows with rosettes of silica and two distinct rows of stomata | ridges with two rows distinct silica tubercles or without silica tubercles
furrows with rosettes of silica and two distinct rows of stomata |
| Strobilus | with 3 whorls of sporophylls
sporangia opening
chlorophyllous, spherical, elater-bearing | with (3-) 4 - 5 (-6) whorls of sporophylls
sporangia not opening
a-chlorophyllous, irregularly shaped, without elaters | with (4-) 5 (-6) whorls of sporophylls
sporangia opening
chlorophyllous, spherical, elater-bearing |

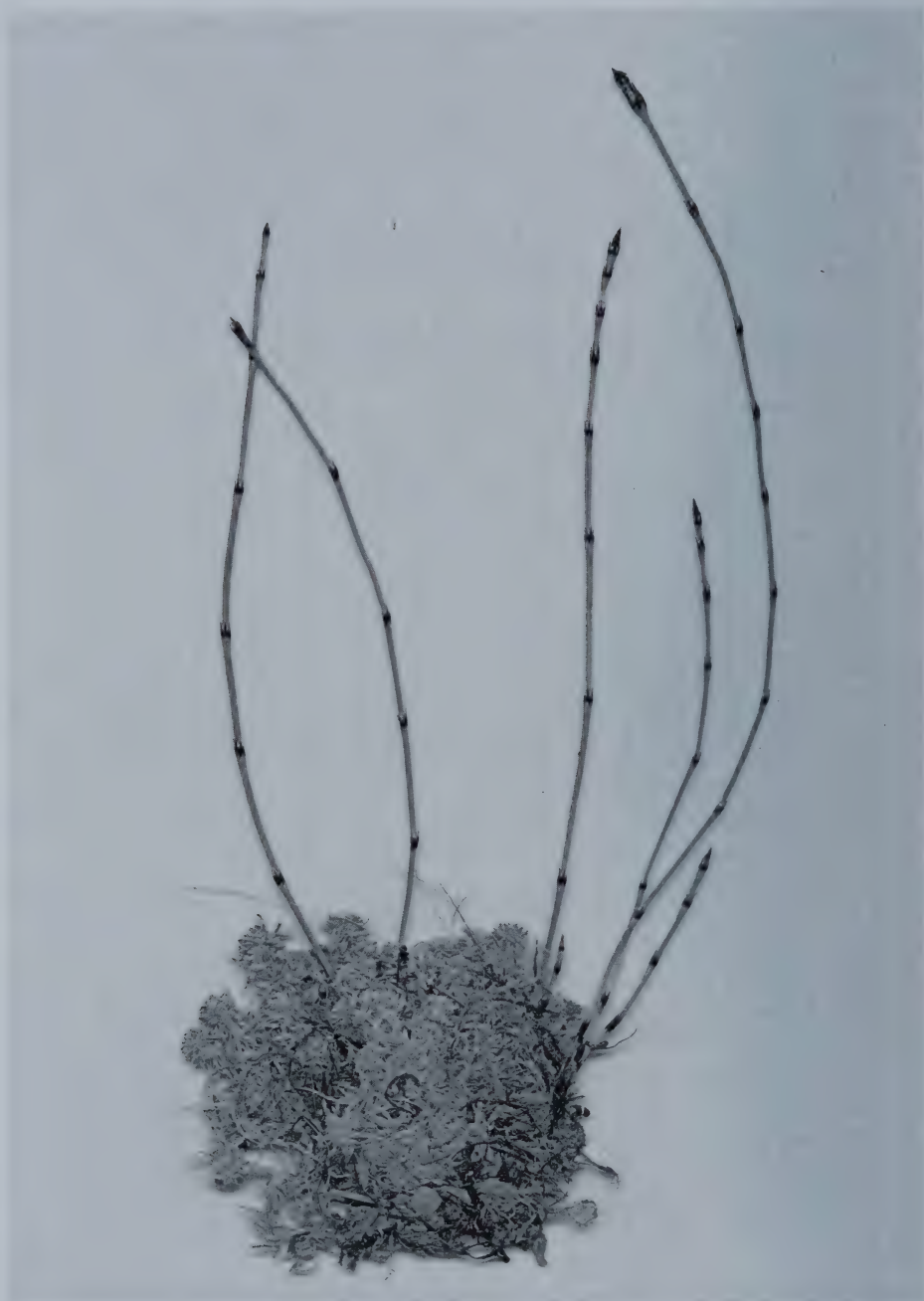


FIG. 1. Type specimen of *E. x lapponicum* (Sweden, Lycksele Lappmark: Artfjället, Gausjosjön, near Strimasund, wet marsh on the eastern shore of Över-Uman, 26 km north of Hemavan, Storums kommun).

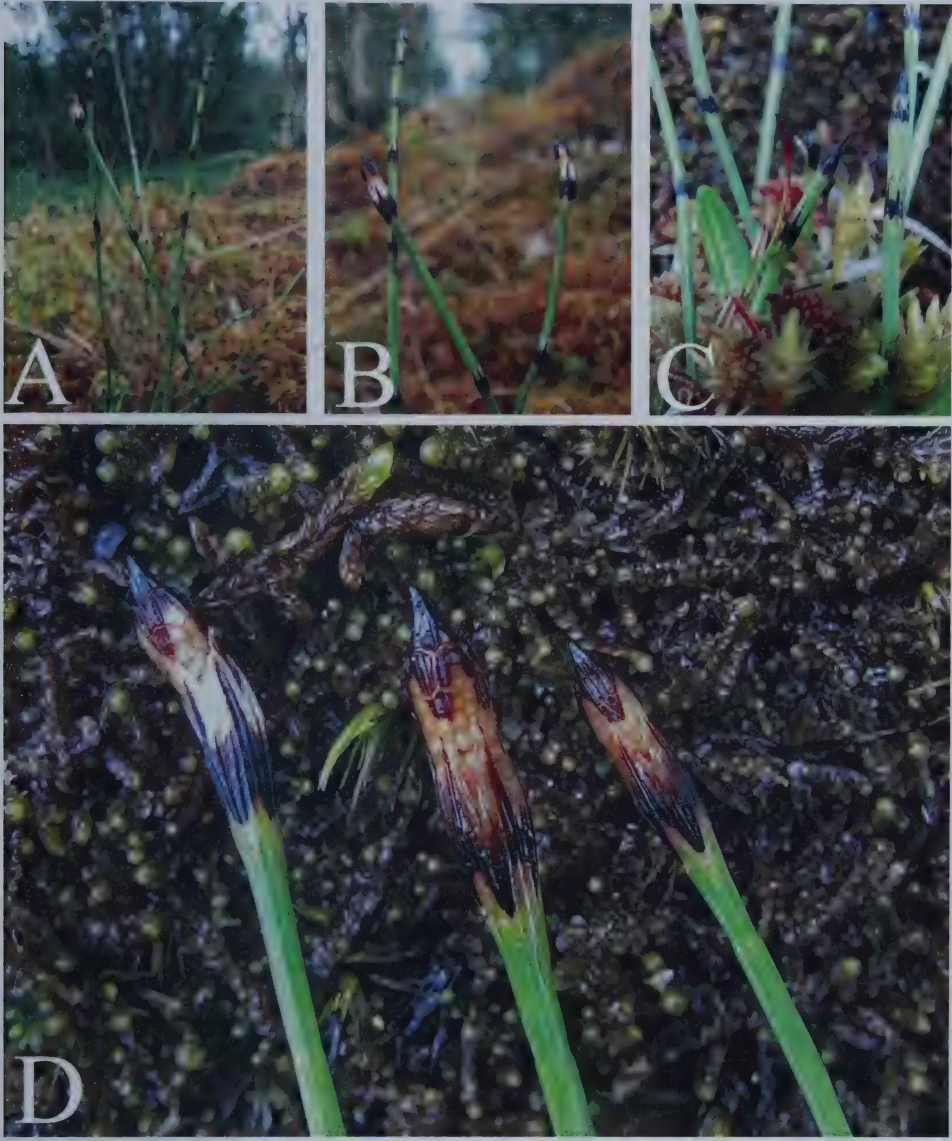


FIG. 2. A–D. *E. ×lapponicum* at Gausjosjön, Lycksele Lappmark, Sweden, 16.07.2014. A and B. Growth habit and shoots. C. Young and emerging shoots showing characteristic long, black sheath teeth. D. Typical small and stunted strobili.

DISCUSSION

Hauke (1963) states, that the hybrid between *E. scirpoides* and *E. variegatum* should show exact intermediate conditions in a measurable sense. As we present here, this is rendered difficult to assess by the high degree of macro- and micromorphological variability in hybrids within subgenus *Hippochaete*. Furthermore, Hauke's statement is not supported by micromorphological

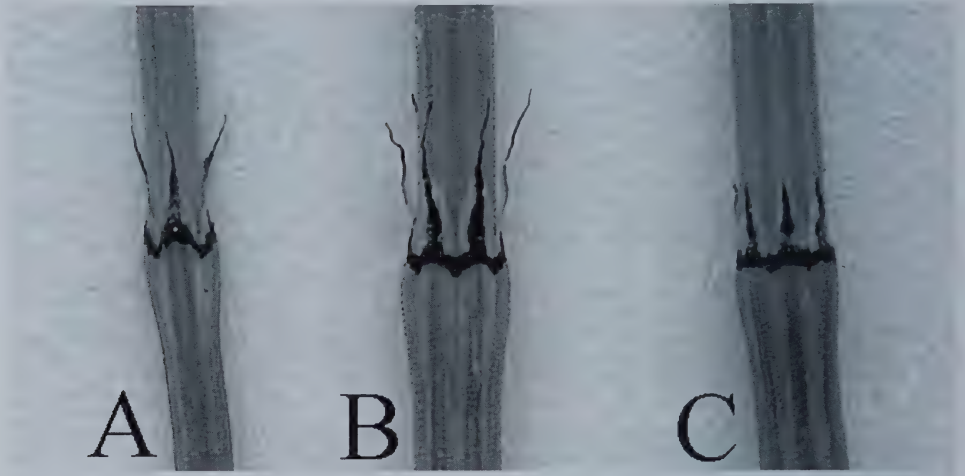


FIG. 3. Nodal sheaths of *E. x lapponicum* (B) and its parent species *E. scirpoides* (A) and *E. variegatum* (C), showing intermediate sheath teeth morphology.

studies in subgenus *Equisetum* (Lubienski, 2010; Lubienski and Dörken, 2013). These studies have shown strong discrepancy between macromorphological and micromorphological characters in some hybrid taxa. Additionally, most quantitative characters in the genus vary with stem size and therefore are not strong criteria for determination.

The high degree of morphological and anatomical similarity (Hauke, 1963) to *E. variegatum* presumably was the reason why botanists hesitated to realize that the nordic *variegatum*-like plants with abortive spores were in fact

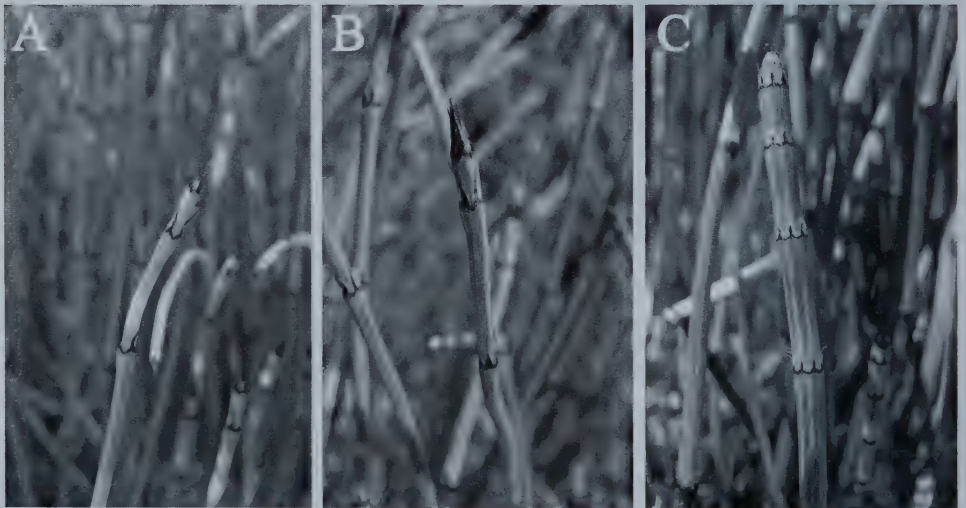


FIG. 4. Emerging shoot apices of *E. x lapponicum* (B) and its parent species *E. scirpoides* (A) and *E. variegatum* (C), showing intermediate sheath teeth morphology.

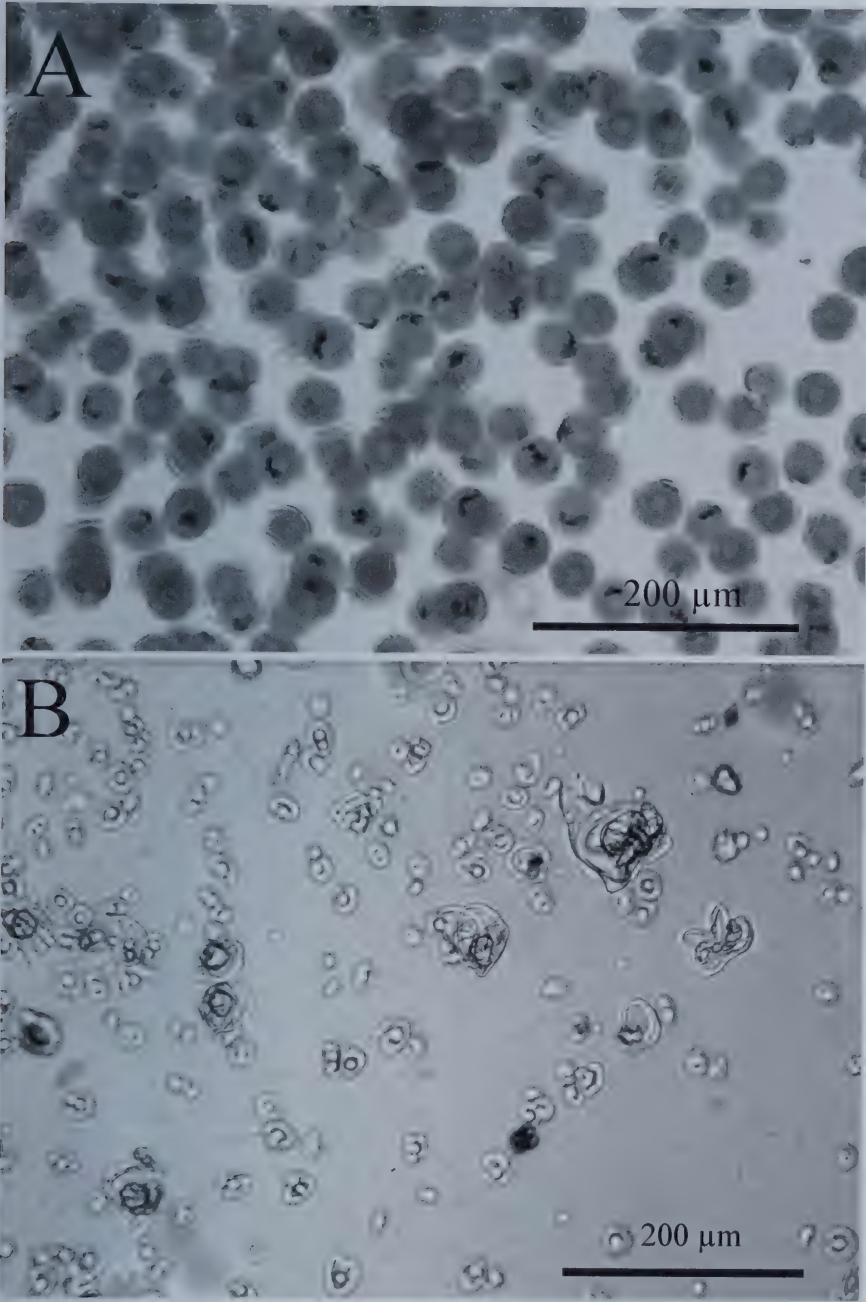


FIG. 5. A–B. A. Well formed, elater bearing, chlorophyllous, and viable spores of *E. scirpoides*. B. Malformed, elater lacking, achlorophyllous, aborted spores of *E. x lapponicum*.



FIG. 6. Potential chlorophyllous diplospore (see arrow) of *E. x lapponicum*.

representatives of a hybrid between *E. scirpoides* and *E. variegatum*. Although intermediate in macromorphology, the small size of both parent species makes this less obvious. Both lack further exclusive and differentiating characters with the exception of the sheath teeth number and character in addition to different growth habits. Micromorphology of the shoots is especially similar in both species (Figs. 7 A–D, Table 1). The existence of very small *E. variegatum* plants, even smaller than *E. x lapponicum* and sometimes coexisting with “normal” *E. variegatum* in nature, further complicates the situation. Cultivation experiments however have shown that these clones retain their small 4-angled shoot morphology in cultivation and produce well-formed, viable spores. Additionally they never have the characteristic long dark-awned sheath teeth like *E. scirpoides* and *E. x lapponicum*. We have never observed hybrid plants growing intermixed with such different forms of *E. variegatum*.

Spore abortion is a very strong indicator for hybridization in ferns. Why Hauke (1963), whose monographic work in *Equisetum* is still unsurpassed, failed to conclude, that those plants with abortive spores in fact are hybrids remains unclear. His explanation for the existence of sterile clones of *E. variegatum* and spore abortion is focused on the climatic conditions in the northern latitudes, where such plants have been found (Hauke, 1963). However, sterile and fertile clones are frequently found side-by-side, sterile clones are absent from regions where *E. scirpoides* does not occur, and the

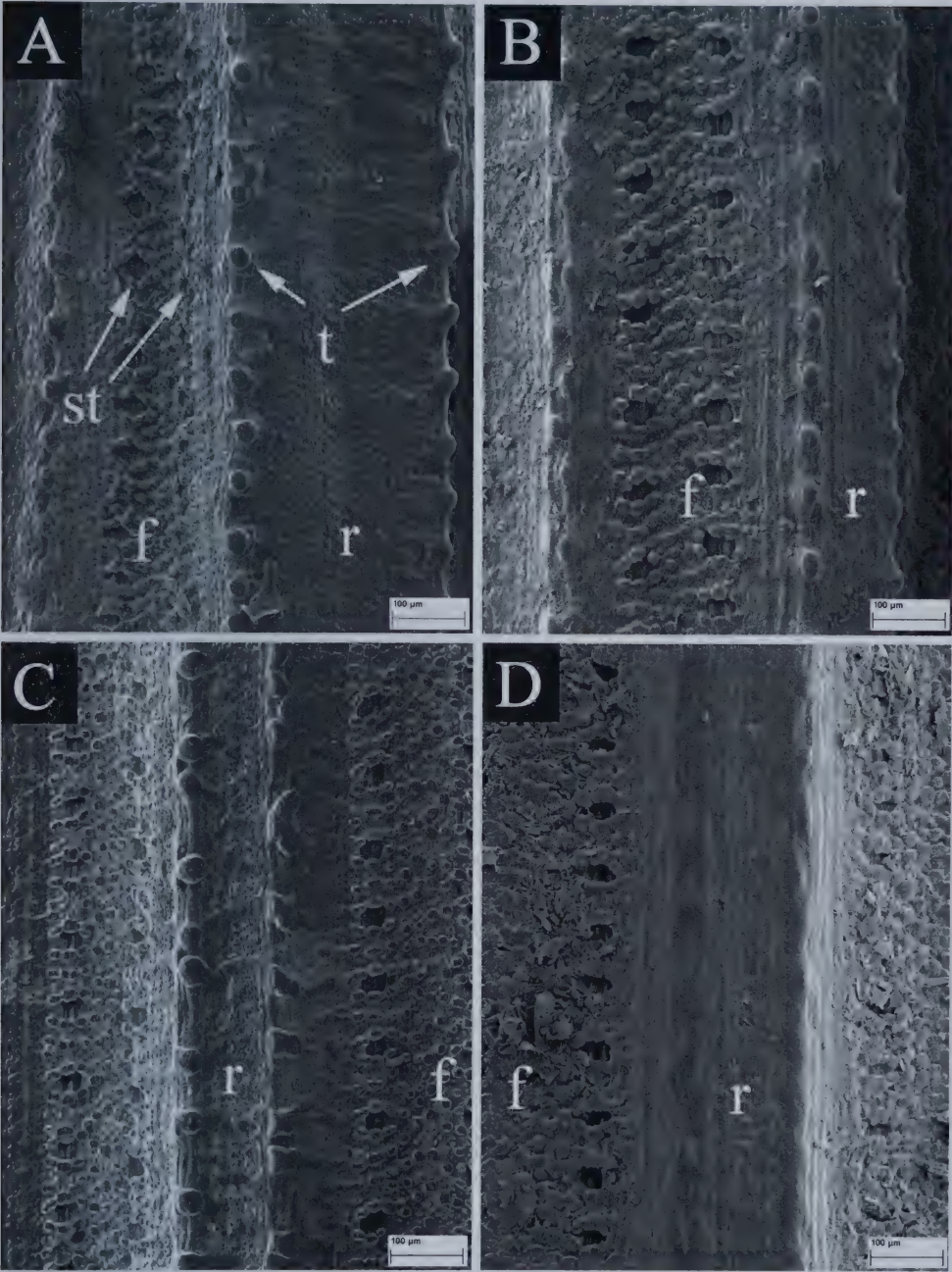


FIG. 7. A–D. SEM images of the micromorphology of the main shoot internode of *E. scirpoides* (A), *E. x lapponicum* (B), and *E. variegatum* (C–D). Ridges (r), furrows (f), stomata (st), silica tubercles on the ridges (t).



FIG. 8. Reticulate evolution in *Equisetum* subgenus *Hippochaete*. Species (black), diploid hybrids (grey), and triploid hybrids (1-3).

phenomenon is not known from other boreal *Equisetum* species. Additionally, spore abortion in *E. ×lapponicum* occurs constantly under controlled conditions in cultivation.

With reference to the number of records in literature and the distributional data for *E. variegatum* with abortive spores shown in Hauke (1963) for North America and Northern Europe, one might presume that *E. ×lapponicum* is much more frequent all over the holarctic. An early reference for the European arctic was given in Holmberg (1920). He worked with several records for Sweden, but finally rejected the existence of this hybrid and placed the specimens in question with small forms of *E. variegatum*, which display a decreased spore production not unusual in Northern Scandinavia. Hiitonen (1933) listed *E. scirpoides* × *E. variegatum* as questionable without any further information, while Hylander (1953) mentioned it for Sweden using Rothmaler's epithet *arcticum*, the record reappeared in Almquist and Björkman (1960). Meusel *et al.* (1971) illustrated a specimen of *E. ×arcticum* from Torne Lappmark in Sweden, however its identity cannot be confirmed due to the imaging technique. Borg (1967) reported his own revisions of material from Fennoscandia and concluded that all specimens are small variants of *E. variegatum*. Øllgaard and Tind (1993) and Øllgaard (2000) later followed this appraisal.

Concerning North America, there is a record of *E. ×arcticum* given in Cody and Britton (1989) for the Canadian Northwest Territories (District of Mackenzie) again using the invalid name of Rothmaler (1944). This record was taken up later by Hauke (1993) although he had rejected the existence of such a hybrid earlier (Hauke, 1963).

Equisetum \times *lapponicum* represents the missing link in a worldwide hybridization scheme within subgenus *Hippochaete*, underlining the high degree of reticulation in this ancient group of plants. Every species in the subgenus is involved in at least one hybridization event over the complete natural range of the members of this group comprising South, Central and North America, Europe, Eurasia, and Africa (Fig. 8). Evolutionary processes reached an extraordinary level in central Europe, where the production of diplospores in the diploid hybrids has led to the formation of three different triploid cytotypes, two of which interpreted as backcrosses and one as a triple hybrid (Bennert *et al.*, 2005; Lubienski and Bennert, 2006; Lubienski *et al.*, 2010). Interestingly, diplospores could be observed in *E.* \times *lapponicum*, too (Fig. 6) which makes a similar scenario of hybridization between the three northern species *E. scirpoides*, *E. variegatum*, and *E. hyemale* possible, at least in theory.

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***Selaginella pectinata* Resurrected — the Correct Name for an Unusual Endemic Spike Moss from Madagascar**

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ABSTRACT.—We review the nomenclature of *Selaginella polymorpha* Badré, endemic to Madagascar, and conclude that this name is illegitimate by superfluity under the International Code of Nomenclature. The name should be rejected and replaced by the older *S. pectinata* Spring, coined in 1843.

KEY WORDS.—lycophytes, nomenclature, *Selaginella polymorpha*, Selaginellaceae

Few Articles in the International Code governing naming of plants (McNeill *et al.*, 2012) have been as ignored, or misunderstood, as Art. 58:

58.1. If there is no obstacle under the rules, the final epithet in an illegitimate name may be re-used in a different name, at either the same or a different rank; or an illegitimate generic name may be re-used as the epithet in the name of a subdivision of a genus. The resulting name is then treated either as a replacement name with the same type as the illegitimate name (Art. 7.4; see also Art. 7.5 and Art. 41 Note 3) or as the name of a new taxon with a different type. Its priority does not date back to the publication of the illegitimate name (see Art. 11.3–11.4).

- *Ex.1.* The name *Talinum polyandrum* Hook. (1855) is illegitimate under Art. 53.1, being a later homonym of *T. polyandrum* Ruiz & Pav. (1798). When Bentham, in 1863, transferred *T. polyandrum* Hook. to *Calandrinia*, he called it *C. polyandra*. This name has priority from 1863, and is cited as *C. polyandra* Benth., not *C. polyandra* (Hook.) Benth.

In essence, Article 58.1 (and its Example 1) allows the re-use of epithets previously adopted for an illegitimate name in a different genus, that is, a “replacement name” (see also Art. 6.11, and examples); this presumes there is no impediment to such use (i.e., a pre-existing homonym does not compete). The new name is based on the previously illegitimate one, is linked nomenclaturally, and consequently has the same type. The new name dates from (has priority beginning with) the new nomenclatural act, and not the date

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of publication of the linked, older, illegitimate name. Moreover, the replacement name has, as sole author, the name of the person who adopts the same epithet used as part of the earlier illegitimate name, i.e., it does not include the author who first described the illegitimate name.

The example of interest to us involves the name of a species of spike moss, *Selaginella*, from Madagascar, a species that was given a new name (*Selaginella polymorpha* Badré) by Badré (in Stefanović *et al.*, 1997) because he believed that the previously applied name was illegitimate, and no other available name at species rank existed. *Selaginella polymorpha* is also the name used for this species in recent phylogenetic and classificatory studies of Selaginellaceae (Zhou *et al.*, 2015; Zhou and Zhang, 2015). The reason for coining this new name, by Badré, was that the previously applied basionym, *Lycopodium pectinatum* Willd. (1810), was a later homonym of *L. pectinatum* Lam. (1792), a different species, from the Mascarene Islands; this species of Lamarck is now considered a synonym of *Selaginella concinna* (Sw.) Spring (see Reed, 1965–66). What Badré did not realize was that Spring's nomenclatural act, transferring (renaming) the illegitimate *Lycopodium pectinatum* Willd. (1810) as *Selaginella pectinata*, in 1843, effectively resurrected the illegitimate epithet *pectinata*, creating a replacement name (*nom. nov.*) for the species, with priority beginning in 1843. (For dates of publication of the Spring names, first published in journals and later issued as separates, see Stafleu and Cowan, 1985). Thus, *Selaginella pectinata* Spring then became the earliest available name for the taxon at species rank. The circumscription given by Badré (in Stefanović *et al.*, 1997) is accepted here. This interpretation of the Code, applying Art. 58.1, makes *Selaginella polymorpha* Badré an unnecessary, superfluous new name for the same taxon. The synonymy for the species, then, is as follows:

- Selaginella pectinata* Spring, Bull. Acad. Roy. Sci. Bruxelles 10(1):146. 1843, *nom. nov.* for *Lycopodium pectinatum* Willd., Sp. Pl., ed. 4. 5(1):44. 1810, *nom. illeg.*, *non* Lamarck (1792: 651). – *Selaginella polymorpha* Badré, Fl. Madagasc. fam. 14:25. 1997, a *nom. nov.* but *superfl.* for *Lycopodium pectinatum* Willd. – Lectotype (here designated): “America meridionale?” [protologue], “Habitat in Madagascar” [specimens], B-W 19400-02 0.
- Lycopodium laevigatum* Lam., Encycl. 3:652. 1792, *nec* Willdenow (1810:45). – *Stachygynandrum laevigatum* (Lam.) P. Beauv., Prodr. Aethéog. 110. 1805. – *Selaginella laevigata* (Lam.) Baker, Gard. Chron. 47:1190. 1867, *nom. illeg.*, *non* (Willd.) Spring (1840: 125), which is *Selaginella willdenowii* (Desv. ex Poir.) Baker, according to Alston (1934, 1945). – Lectotype (here designated): Commerson *s.n.* [publication], Madagascar (P-LA, Lamarck Herbier 127, bar code P00565102, with original label “ex ins. Madagasc”, fertile specimen, presumably in Lamarck's handwriting); isolectotypes: BM (not seen, no images online), P-LA (Lamarck Herbier 128, bar code P00565103, sterile specimen, annotated by Morton, 1967); both P-LA sheets with typewritten labels as *Selaginella polymorpha* Badré, *nom. nov.*

Selaginella pectinata Spring var. *curvifolia* Spring, Mém. Acad. Roy. Sci. Belgique 24:167. 1849 [Monogr. Fam. Lycopod. 2:167. 1850]. – Type: collector unknown, Madagascar (P, not seen, no image online).

Selaginella laevigata (Lam.) Baker var. *brachystachys* Hieron., in Engler and Prantl, Nat. Pflanzenfam. 1(4):707. 1901 (1902). – Type: collector unknown, Madagascar (B? not seen, no image online).

Selaginella polymorpha Badré is a *nom. nov.* for, and with the same type as, *Lycopodium pectinatum* Willd., but superfluous, since an earlier name, *Selaginella pectinata* Spring, was available. We designate sheet B-W 19400-02 0, the more ample specimen, as lectotype; an isolectotype is B-W 19400-01 0. The renaming of this species by Spring (1843) is without description, and so the name is based entirely on *Lycopodium pectinatum* Willd. (1810); hence the type is the specimen designated by Willdenow. Spring also cited *Lycopodium laevigatum* Lam., Encycl. 3:652, 1792, as a heterotypic synonym, a taxonomy accepted by Badré (in Stefanović *et al.*, 1997). *Lycopodium pectinatum* Lam. (the name that makes *L. pectinatum* Willd. a later homonym and illegitimate), was said to “croît naturellement aux Indes orientales, à l’isle de France & dans les pays chauds de l’Amérique”; it is now considered to be a species from Mauritius and Réunion, and was treated as a synonym of *Selaginella concinna* (Sw.) Spring (Badré, 2008).

Selaginella pectinata belongs to a small, apparently isolated group of *Selaginella* comprising about eight species, as shown by phylogenetic analyses of DNA sequence data, as well as morphological studies (Schulz *et al.*, 2013; Korall and Kenrick, 2002; Weststrand and Korall, unpubl.; Zhou *et al.*, 2015; corresponding to *Selaginella* sect. *Lyallia* in Zhou and Zhang, 2015); the clade is restricted to the southern hemisphere, in the Old World, predominantly in austral areas – South Africa, Madagascar, Comoros, and Australia, also one species in New Guinea. Currently known species in this group are *S. aboriginalis* C. Schulz & Homberg (from Queensland, Australia; Schulz *et al.*, 2013); *S. gracillima* (Kunze) Spring ex Salomon (syn.: *S. preissiana* Spring; from western Australia); *S. lyallii* (Hook. & Grev.) Spring (from Madagascar); *S. moratii* W. Hagemann & Rauh (from central Madagascar); *S. pectinata* Spring (from Madagascar, Comoros Islands); *S. pygmaea* (Kaulf.) Alston (from South Africa); *S. royenii* Alston (from New Guinea); and *S. uliginosa* (Labill.) Spring (from eastern Australia, Tasmania, Lord Howe Island, and other offshore islands). Three of these species were included in subg. *Ericetorum* by Jermy (1986): the mainly Australian *S. uliginosa* (the type) and *S. gracillima*, and the South African *S. pygmaea*.

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Identifying a Cryptic *Adiantum* Population through DNA Barcoding

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ABSTRACT.—Populations of *Adiantum* growing on serpentine barrens in Pennsylvania and Maryland have been identified as *A. aleuticum* (Rupr.) C.A.Paris (= *A. pedatum* L. var. *aleuticum* Rupr.). This species, and the allopolyploid *A. viridimontanum* C.A.Paris, are known from serpentine in New England and Canada. A phylogenetic analysis based on two plastid markers showed that the Pennsylvania and Maryland populations are *A. pedatum sensu stricto* rather than *A. aleuticum* or *A. viridimontanum*.

KEY WORDS.—*Adiantum aleuticum*, *Adiantum pedatum*, DNA barcoding, serpentine, *rbcL*, *trnL-F*

The *Adiantum pedatum* species complex is a group of temperate ferns found across North America and in northern Asia from the Pacific west to the Himalayas (Lu *et al.*, 2011). Chromosome counts (Nakato and Kato, 2005) and plastid phylogenies (Lu *et al.*, 2011) indicate the presence of cryptic taxa within the complex in Asia, which have not yet been delineated. In North America, the West Coast populations of the complex were recognized as distinct by Presl (Presl, 1836) and described by Ruprecht as *Adiantum pedatum* var. *aleuticum* (Ruprecht, 1845). Ruprecht's original diagnosis noted the similarity between the western material he assigned to var. *aleuticum* and specimens of *A. pedatum* collected from Newfoundland and the barrens of Pennsylvania (Ruprecht, 1845). Certain populations on serpentine from Mt Albert, Quebec were assigned to *A. pedatum* var. *aleuticum* in 1905 (Fernald, 1905), and additional serpentine populations in Newfoundland, Quebec, and Vermont, though not as morphologically distinctive as the Mt Albert material, were likewise assigned to the variety soon after (Mousely, 1923). Various classifications of serpentine populations and non-serpentine western populations were proposed (Calder and Taylor, 1965; Cody, 1983) until 1991, when Paris described an allotetraploid, *Adiantum viridimontanum*, between the eastern and western taxa and elevated the western taxon to species level as *Adiantum aleuticum* (Paris 1991). However, her circumscription for that taxon, based on a morphometric analysis, differed in certain characters from earlier diagnoses. In particular, she found the uprightness of the plant, previously considered diagnostic, to be unreliable in distinguishing the species within the complex. In light of these changes in

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diagnosis, material historically determined as *A. pedatum* var. *aleuticum* is not necessarily referable to *A. aleuticum*. Since *A. aleuticum* appears to be limited in eastern North America to serpentine substrates (Paris, 1991) and perhaps dolomite (Cody, 1983), an accurate delineation of its range is important for understanding substrate preferences within the *A. pedatum* species complex.

Adiantum pedatum from the State Line serpentine barrens in Pennsylvania and Maryland has historically been described as a “serpentine form” (Overlease, 1986), “dwarf form” (Wherry, 1963), or attributed to *A. pedatum* var. *aleuticum* (Wherry, 1975). Presumably as a result of the latter report, *A. aleuticum* has been reported among the flora of Pennsylvania and Maryland (Paris, 1993; Rhoads and Block, 2007). This assignment has not always been accepted by local botanists (Wes Knapp, pers. comm.) and a plant from the Goat Hill serpentine barrens was found to be *A. pedatum* s.s. (Pennsylvania Rare Plant Forum, 2009; Jun Wen, pers. comm.). In light of the subtle morphological differences between taxa of the *A. pedatum* complex, a molecular analysis of material collected from the State Line barrens was carried out to identify their species. The publication of an Asian–North American phylogeny of the complex during the course of the present study (Lu *et al.*, 2011) provided a basis for genetic comparison of these samples to *A. pedatum* s.s. and both eastern and western populations of *A. aleuticum*.

MATERIALS AND METHODS

Collection.—Representative specimens of *Adiantum* were collected from the following serpentine barrens: Bald Hill (Harford Co., MD), Goat Hill (Chester Co., PA and Cecil Co., MD), Pilot (Cecil Co., MD), and Nottingham (Chester Co., PA). In total, 12 vouchered samples were collected and deposited at PH: 8 from Goat Hill, 2 from Pilot Barrens, 1 from Bald Hill, and 1 from Nottingham County Park (Table 1). Habitats sampled included shaded areas along streams, steep banks in power line cuts, and the toe of a serpentine talus slope in an old mine. A historical population at Cedar Barrens (Chester Co., PA) is considered extirpated; a specimen at PH from this population (F.W. Pennell 3939, 27 June 1912) was annotated “not the serpentine fern” by Wherry, Paris concurring. Reports of *Adiantum pedatum* (no variety specified) on serpentine at Soldier’s Delight (Baltimore Co., MD) could not be confirmed.

DNA extraction, PCR, and sequencing.—Total DNA was extracted from 10–20 mg of silica-dried leaf tissue. Extraction was performed with a Qiagen DNeasy Plant Mini Kit, following the manufacturer’s protocols. The dried plant material was ground by hand directly in the kit’s lysis buffer. The plastid loci *rbcL* and *trnL-F* were amplified by PCR using the primers 1FN (de Groot *et al.*, 2011) and 1379R (Wolf *et al.*, 1999) for *rbcL* and primers FERN-1 (Trewick *et al.*, 2002) and “F” (Taberlet *et al.*, 1991) for *trnL-F*. Each PCR was performed on a 40- μ L scale with 20 μ L of TopTaq Master Mix (2 \times), 2 μ L of each primer (10 μ M), 1 μ L of the appropriate DNA template (7.5–59 ng), and 15 μ L of DNase-free H₂O. The PCRs were performed under the following conditions: 1 cycle at 95°C for 1 minute, then 35 cycles at 95°C for 1 minute, 51°C for 1 minute, and 72°C for

1 minute and 20 seconds, then 1 cycle at 72°C for 10 minutes. In order to fully remove the PCR primers, 4 µL of ExoSAP-IT was added to each reaction, which was then incubated at 37°C for 1 hour and inactivated at 85°C for 15 minutes. After the digestion of the primers, samples were purified with a QIAquick PCR Purification Kit, following the manufacturer's protocols. Purified PCR reactions were sequenced using an ABI Prism 3130XL Genetic Analyzer and a BigDye Sequencing Kit at the University of Delaware sequencing facility.

Phylogenetic analysis.—The 12 experimental samples were combined with 17 samples for which sequence data was available in GenBank: 10 of *Adiantum pedatum*, 6 of *A. aleuticum*, and 1 of *A. viridimontanum* (Appendices 1 and 2). One of these *A. aleuticum* samples was taken from the eastern populations of the species, in Quebec. Sequence alignment was performed in MEGA 6.06 (Tamura *et al.*, 2013). The *rbcL* and *trnL-F* sequences were separately aligned and trimmed to the length of the shortest included sequence (1176 and 831 bp, respectively). The partition homogeneity test in PAUP* 4.0b10 (Swofford, 2003) did not indicate a significant difference between the datasets corresponding to the two loci, so the two were combined in a single matrix. The combined dataset contains 2007 sites, of which 26 were variable and 14 were parsimony-informative. Phylogenetic trees were constructed using maximum parsimony (MP) in PAUP*. The MP analysis was performed using the heuristic search option with 1,000 random addition sequence (RAS) replicates, swapping branches with the TBR algorithm and with Multrees enabled. Gaps were treated as missing data. Bootstrap analysis was performed with 1000 replicates. The final dataset and trees were deposited in TreeBase (accession S17250).

Morphology.—To ensure that the contemporary samples of *Adiantum* were representative of past reports of *A. pedatum* var. *aleuticum*, historical material was sought at PH and DOV. PH was chosen in part as the repository of the collections of Francis W. Pennell, who collected extensively on the State Line Barrens from about 1910 to 1929 (Pennell, 1930). The following specimens at PH were annotated by E. T. Wherry as “serpentine form” (John Kunsman, pers. comm.): E. T. Wherry, *s.n.*, 7 September 1960; F. W. Pennell 10967, 21 September 1920; F. W. Pennell 16188, 27 June 1933; and E. T. Wherry *s.n.*, 18 June 1938. All of these collections were made either in Chester County, at the north edge of Goat Hill on the steep banks above Octoraro Creek (“southeast of Lees Bridge”), or immediately across the creek in Lancaster County, near the “Wood Chromite Mine” and “south of Wrightsdale”. Unfortunately, these specimens could no longer be found at PH. An additional Wherry collection—E. T. Wherry, *s.n.*, October 20, 1974, from the Cedar Hill serpentine quarry south of Wrightsdale—was located at PH and examined; Wherry did not determine it to a particular variety or form. Digital images of F. W. Pennell 19067, and another specimen from serpentine “along Octorara Creek, Lees Bridge,” F. W. Pennell *s.n.*, June 22, 1929 were subsequently obtained from NY.

The last three specimens, together with the twelve vouchers of this study, were examined with respect to the characters used for diagnosis in *Flora of*

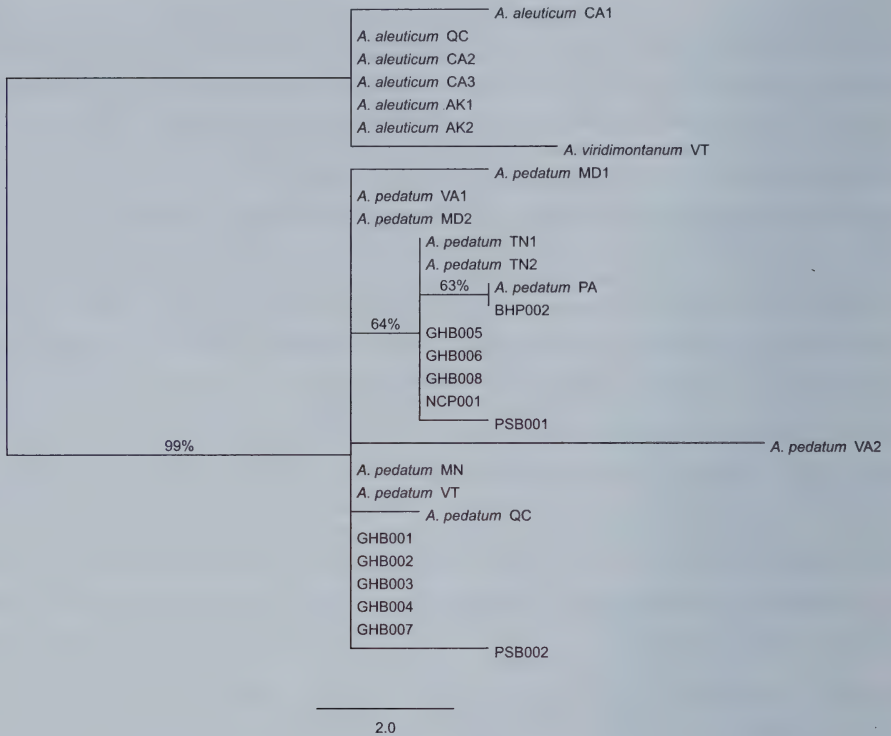


FIG. 1. One of the most parsimonious trees of sequences included in this study, in units of inferred changes. Bootstrap support values >50% are indicated. Sequence and voucher data are provided in Appendices 1 and 2.

North America (Paris, 1993). In *A. pedatum*, the ultimate segments are less than 3.2 times as long as broad (rather than more than 3.2 times as long as broad), the apices of the segments have rounded, crenulate, or crenate-denticulate lobes (rather than sharply denticulate lobes) with shallow sinuses 0.1–2(–3.7) mm deep (rather than 0.6–4 mm), and the segments are borne on stalks generally 0.6–0.9 mm long (rather than 0.6 mm or less). Measurement of characters followed Fig. 1 of Paris and Windham (Paris and Windham, 1988). As described therein, the four ultimate segments at the middle of the longest penultimate segment were measured and averaged for each specimen, with the following caveats: GHB 8 (C. Hoess 10) consisted of one penultimate segment only, on which measurements were performed, while only three and one segments, respectively, could be measured on BHP 2 (C. Hoess 5 & C. Watson) and GHB 6 (C. Hoess 7).

Spores from three specimens collected at Goat Hill and one collected at Bald Hill were mounted in glycerol jelly on glass slides. These slides were imaged using a Meiji Techno 5210H microscope fitted with an OptixCam Summit 9.0 USB camera at 200× magnification, calibrated with a hemocytometer, and the diameters of 24–38 spores per slide were measured.

TABLE 1. Population name, sample abbreviation, location, and geographic coordinates for samples used in this study.

| Population name | Abbreviation | Location | Geographic coordinates |
|----------------------------|--------------|-----------------|------------------------|
| Bald Hill Powerline 2 | BHP 2 | Harford Co., MD | 39.6935° N 76.2315° W |
| Goat Hill Barrens 1 | GHB 1 | Chester Co., PA | 39.7255° N 76.0800° W |
| Goat Hill Barrens 2 | GHB 2 | Chester Co., PA | 39.7278° N 76.0748° W |
| Goat Hill Barrens 3 | GHB 3 | Chester Co., PA | 39.7238° N 76.1024° W |
| Goat Hill Barrens 4 | GHB 4 | Chester Co., PA | 39.7327° N 76.0933° W |
| Goat Hill Barrens 5 | GHB 5 | Cecil Co., MD | 39.7211° N 76.0824° W |
| Goat Hill Barrens 6 | GHB 6 | Chester Co., PA | 39.7237° N 76.0784° W |
| Goat Hill Barrens 7 | GHB 7 | Chester Co., PA | 39.7250° N 76.1086° W |
| Goat Hill Barrens 8 | GHB 8 | Chester Co., PA | 39.7225° N 76.0803° W |
| Nottingham County Park 1 | NCP 1 | Chester Co., PA | 39.7314° N 76.0396° W |
| Pilot Serpentine Barrens 1 | PSB 1 | Cecil Co., MD | 39.7043° N 76.1899° W |
| Pilot Serpentine Barrens 2 | PSB 2 | Cecil Co., MD | 39.7045° N 76.1901° W |

RESULTS

The MP analysis yielded two trees of length 29, consistency index (CI) of 0.93, and retention index (RI) of 0.97. Both trees included a clade that contained all samples from this study and all sequences of *Adiantum pedatum* s.s. from GenBank, and excluded *A. aleuticum* and *A. viridimontanum*. This clade was strongly supported (99%) by the bootstrap analysis. Two subclades, including six of this study’s samples and GenBank sequences of *A. pedatum* s.s. from Pennsylvania and Tennessee, were weakly supported by the bootstrap analysis (62% and 64%); in general, geographic structure was not apparent within the strongly supported clade. A larger dataset with more phylogenetically significant characters would be needed to draw any firm conclusions on the point.

The morphological characters of the twelve samples collected, and the two digitized herbarium specimens, were almost entirely consistent with *Adiantum pedatum*, with the exception of the ultimate segment shape of GHB 3 (C. Hoess 3 & C. Watson), where the average ratio of length to breadth was 3.2, and the average stalk length of NCP 1 (C. Hoess 9 & C. Watson), which was 0.53 mm. GHB 1 (C. Hoess 1 & C. Watson), PSB 2 (C. Hoess 12 & W. Knapp), and Pennell s.n., June 22, 1929, all had average stalk lengths greater than 0.9 mm.

The average spore diameters (\pm 1 s.d.) were $38.93 \pm 2.87 \mu\text{m}$ for the Bald Hill specimen and $34.92 \pm 3.05 \mu\text{m}$, $38.11 \pm 3.82 \mu\text{m}$, and $37.15 \pm 3.23 \mu\text{m}$ for the Goat Hill specimens. These measurements are consistent with the mean size of $37.03 \mu\text{m}$ reported for diploid *A. pedatum*, rather than the mean size of $51.36 \mu\text{m}$ reported for tetraploid *A. viridimontanum* (Barrington *et al.*, 1986).

DISCUSSION

While *Adiantum* from the State Line serpentine barrens has often been referred to as *A. pedatum* var. *aleuticum* or *A. aleuticum*, a careful survey of the historical record shows that these labels have not been consistently

applied. Furthermore, the morphology of the *A. pedatum* complex is cryptic and heavily influenced by environmental conditions. While no consistent geographic structure within each species could be discerned from this dataset, *A. aleuticum* and *A. viridimontanum*, including *A. aleuticum* from eastern North America, formed a clade readily distinguished from *A. pedatum* s.s. The failure to locate any *A. aleuticum* among the *Adiantum* sampled from the State Line barrens strongly suggests that *A. aleuticum*, in the presently accepted sense, does not exist in Pennsylvania or Maryland.

Although the plants of the State Line barrens are often somewhat upright, this is due to environmental conditions rather than genetic differentiation from woodland *Adiantum pedatum*; the authors have seen the upright character change dramatically in a single plant during one growing season. In light of their inability to find *A. aleuticum* in a variety of locations from the State Line barrens, the authors recommend that it be removed from the floras of Pennsylvania and Maryland. Specimens referred to that taxon from these states appear to be sun-exposed and dwarfed forms, and indeed, have been identified as such in the past.

While the possibility of *Adiantum pedatum* growing on serpentine has already been suggested by the existence of *A. viridimontanum*, our findings make it clear that *A. pedatum* can survive on exposed serpentine soils, complicating the problem of identification. More work remains to clearly delineate species boundaries in this complex from a morphological perspective.

ACKNOWLEDGMENTS

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APPENDIX 1

The specimens collected during this study are listed in the following format:

Sample abbreviation: collector(s) and collection number, herbarium accession at PH, GenBank *rbcL* accession, GenBank *trnL-F* accession.

GHB 1: C. Hoess 1 & C. Watson, 01115620, KP898416, KP898428.

GHB 2: C. Hoess 2 & C. Watson, 01115621, KP898417, KP898429.

GHB 3: C. Hoess 3 & C. Watson, 01115622, KP898418, KP898430.

GHB 4: C. Hoess 4 & C. Watson, 01115623, KP898419, KP898431.

BHP 2: C. Hoess 5 & C. Watson, 01115624, KP898415, KP898427.

GHB 5: C. Hoess 6, 01115625, KP898420, KP898432.

GHB 6: C. Hoess 7, 01115626, KP898421, KP898433.

GHB 7: C. Hoess 8 & C. Watson, 01115627, KP898422, KP898434.

NCP 1: C. Hoess 9 & C. Watson, 01115628, KP898424, KP898436.

GHB 8: C. Hoess 10, 01115629, KP898423, KP898435.

PSB 1: C. Hoess 11 & W. Knapp, 01115630, KP898425, KP898437.

PSB 2: C. Hoess 12 & W. Knapp, 01115631, KP898426, KP898438.

APPENDIX 2

Sequences retrieved from GenBank, originally published by Lu *et al.* (Lu *et al.*, 2012), are listed in the following format:

Taxon and abbreviation of state or province: *rbcL* accession number, *trnL-F* accession number.

***Adiantum aleuticum* CA 1:** JN052802, JN052908. ***Adiantum aleuticum* QC:** JN052804, JN052831. ***Adiantum aleuticum* CA 2:** JN052806, JN052909. ***Adiantum aleuticum* CA 3:** JN052805, JN052907. ***Adiantum aleuticum* AK 1:** JN119855, JN052921. ***Adiantum aleuticum* AK 2:** JF935362, JF980709. ***Adiantum pedatum* MD 1:** JF935361, JF980708. ***Adiantum pedatum* VA 1:** JN052796, JN052915. ***Adiantum pedatum* MD 2:** JN052795, JN052919. ***Adiantum pedatum* TN 1:** JN052799, JN052912. ***Adiantum pedatum* TN 2:** JN052794, JN052916. ***Adiantum pedatum* VA 2:** JN052792, JN052913. ***Adiantum pedatum* MN:** JN052800, JN052917. ***Adiantum pedatum* PA:** JN052801, JN052911. ***Adiantum pedatum* VT:** JN052798, JN052918. ***Adiantum pedatum* QC:** JN052797, JN052914. ***Adiantum viridimontanum* VT:** JF935347, JF980692.

SHORTER NOTES

***Marsilea owambo*, a New Name for *Marsilea vera* (Marsileaceae).**—The extant species *Marsilea vera* Launert (Launert, Mitt. Bot. Staatssaml. Münch. 3:505–509. 1960) was described from southwestern Africa and is in current use (Gibbs Russell & al, Mem. Bot. Surv. S. Africa 2[1–2]:1–152[pt.1], 1–270[pt.2]. 1987; Hassler & Swale, <http://homepages.caverock.net.nz/~bj/fern/marsilea.htm>—accessed: 30 Jul 2015. 2001). However, there is an earlier validly published fossil species, *M. vera* Jarm. (Jarmolenko, Trudy Sredneaziatsk. Gos. Univ., Ser. 8b, Bot. 28:1–36. 1935), which was established on the fossil foliage remains from the Upper Cretaceous (Turonian) sediments of Kyzyl-Zhar, Karatau ridge, Kyrgyzstan (formerly Kazakh SSR, USSR, Central Asia). Due to the creation of the *International Fossil Plant Names Index* [fossilplants.info] with listing of all fossil plant species, the fact of the homonymy between fossil and extant species was solidly established (IFPNI, 2014-).

Because the later homonym *Marsilea vera* Launert is currently used and does not have any heterotypic synonyms, a new name, *M. owambo*, is here proposed for it.

Marsilea owambo Doweld, nom. nov. for *M. vera* Launert, Mitt. Bot. Staatssaml. Münch. 3: 505. 1960, nom. illeg., non †*M. vera* Jarmolenko, Trudy Sredneaziatsk. Gos. Univ., Ser. 8b, Bot. 28: 6. 1935. TYPE: NAMIBIA. Olukonda: Ovamboland (Ovamboland), 2 Jan 1876, *H. Schinz s.n.* (holotype: B 20 0116365).—ALEXANDER B. DOWELD, National Institute of Carpology (Gaertnerian Institution), 21 Konenkova Street, RUS-127560, Moscow, Russian Federation. E-mail: nicar-sekretariat@yandex.ru; sekretariat@doweld.pro.

OBITUARY

Prof. Dr Bert Hennipman

25th April 1937 – 6th August 2014

When Elbert (Bert) Hennipman died in August last year, he was well into the start of his fourth career.

His first career, starting in 1958, was as a lichenologist, when he took up the study of lichens under the guidance of R.A. Maas Geesteranus, at the then Rijksherbarium in Leiden. This resulted not only in a graduation ‘cum laude’ in 1962, but also in a finely illustrated (by Ed Vijsma) treatment of the Dutch cladonias, a work that has initiated a number of lichenologists and no small number of Dutch field ecologists to lichens. But this was not to be Bert’s future.

His second career started in 1965, when he was told by C.G.G.J. van Steenis to ‘do the ferns’. At the time, pteridology was not represented in Leiden, and Bert undertook his task of establishing a department of pteridology with characteristic enthusiasm. In this, he was helped by G.J. de Joncheere, a retired shipping magnate, who was introduced to ferns by Van Steenis while in Java.

The Rijksherbarium was, at the time, part of the University of Leiden, and Bert’s educational qualities were quickly recognised (by some he is still remembered as one of the best biology teachers of that time), and he was asked to revise the Leiden biology curriculum.

Apart from some spin-off publications, Bert’s major first contribution to pteridology was his world monograph of *Bolbitis*, for which he was awarded the Jesse M. Greenman Award of the American Botanical Society. He continued by taking up the family Polypodiaceae, at the time seriously in disarray. For this, he enlisted the help of a number of enthusiastic MSc students – Marco Roos being one. Together, they produced a systematic account of the staghorns (*Platynerium*), as well as a number of preliminary treatments on smaller genera such as *Lemmaphyllum*, *Belvisia* and *Drymoglossum*. He made major collections in northern Thailand (1966) and central Celebes (1979). By now, he was also firmly committed to new techniques such as electron microscopy, life-cycle studies and new methodologies such as Hennigian phylogenetic analysis. With a passion for teaching, he accepted a position as Professor at Utrecht University in 1980, but continued to be involved in Leiden, where he had a number of PhD students – first Peter Hovenkamp, then Monique Bosman and Gerda van Uffelen. In Utrecht, he continued his collaboration with Marco Roos, now a PhD student working on the drynarioids. The pteridological work during this period resulted in the treatment of Polypodiaceae for the *Families and genera of vascular plants*, and laid the foundation for the treatment of Polypodiaceae in *Flora Malesiana*. The list of co-authors for this flora instalment gives an idea of how many students he managed to inspire and guide. He also persuaded Ed Wiley to spend a sabbatical year in Leiden, and used this occasion to organise a number of meetings that helped spread phylogenetic methodology in the Netherlands.

This second career was cut short when Bert, after a period of infighting in the Utrecht department of biology, was forced to take early retirement in 1991. At the start of his Utrecht career, the first major round of budget cuts of the Dutch universities took place, which resulted in the closing down of the department of systematic zoology of Utrecht University. At that time the Herbarium was not threatened, also because of his recent appointment. But the ambitions of Bert to develop a modern phylogenetic approach did not always match the ideas of other members of the Herbarium, which led to a rather tense and difficult working atmosphere and the establishment of two opposing factions. The board of the faculty was not very resolute in trying to solve the problems. The second major round of university budget cuts, therefore, affected the Herbarium seriously, leading to an almost halving of the staff, especially affecting Bert's research programmes.

However, in line with his fighting spirit he did not take this as a retirement, but as the start of a third career, which again he undertook with gusto, only temporarily hampered by his Non-Hodgkin cancer, which, with the same spirit, he was able to overcome. Relieved from University duties, he could now give free rein to his entrepreneurial spirit. Together with a number of fern growers and with Clive Jermy and Trevor Walker, he entered into a start-up (as we would now call it), with the aim of commercialising their knowledge of ferns, fern ecology and fern reproduction. This quickly expanded to include other groups of plants, and in no time, Bert was travelling the world to locate interesting material for cultivation. His approach was innovative in that he used phylogenetic evidence to focus attempts on related taxa when looking for sources for the expansion of genetic variation. He also specifically searched for plant populations growing in biotopes with physical conditions comparable to the Dutch climate. This was a departure from the way genetic diversity was generally collected in commercial circles, which was mostly by randomly picking from existing collections in gardens. He organised his own laboratory in the attic of his house and created a number of parental lines *in vitro* in order to improve the genetic variation in *Hippeastrum* cultivars. The most successful project was the introduction of genes of *Hippeastrum cybister* into the existing commercial breeder's stock, which led to a new group of cultivars with narrow-tepaloid flowers called the *Hippeastrum* Spider Group. This cultivar group is now well established in commerce.

In the meantime he did not let go of ferns, and was officer of the Nederlandse Varenvereniging (Dutch Fern Society) from 1989 to 1996. He remained a member of the British Pteridological Society until 2003, having joined in 1971. He finally relinquished ferns when he continued this entrepreneurial career in 2002 as Director of B-ware Research centre, a spin-off of the Radboud University in Nijmegen. He was instrumental in founding this company and led it as Director until 2008.

Now with time on his hands, he started a fourth career with his usual energy and drive. He took painting lessons, and the last time we (PH, GvU) met him was on the occasion of the first exposition of his paintings. These were not the

usual insipid amateur paintings of someone who took up painting just as a pastime after retirement. Well-trodden paths were never Bert's way. With his eye for detail he was able to turn small bits of tree bark, twigs and other rather commonplace natural objects into bold and strikingly original paintings.

But we cannot tell where this talent would have taken him. His body, prematurely aged by his episode of chemo-treatment, finally failed him on 6th August 2014.

We celebrated his life with friends, family and colleagues on 14th August 2014.

Peter Hovenkamp, Wilbert Hetterscheid, Marco Roos, Gerda van Uffelen

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OBITUARY

Anthony Clive Jermy

3rd July 1932 – 25th July 2014

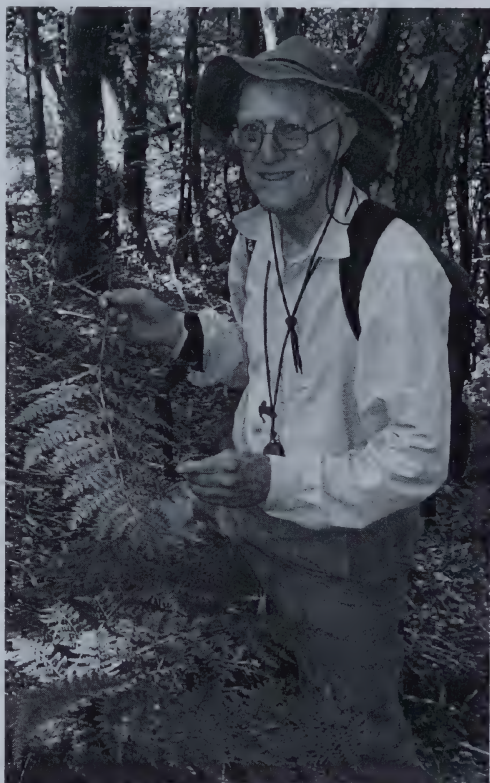
Clive Jermy, past President and Honorary Member of the BPS and former Head of the Fern Section at the Natural History Museum, was a prolific letter writer and a list of his correspondents would read like a ‘who’s who’ of British botany, international pteridology and plant conservation.

Tony, as his family knew him, was born in Strumpshaw, a small Norfolk village east of Norwich. During his youth he spent many hours collecting plants in the surrounding marshes and meadows and the house was always full of jars of plant specimens. He attended the City of Norwich Grammar School and during this time got to know and work with notable Norfolk botanists, Ted Ellis and Joyce Lambert. He went on to gain his BSc at University College, London and thence to Leicester University to start a PhD on *Carex*. However, National Service loomed and Clive (he had adopted his middle name to avoid confusion with other Tonys in his class) opted to serve as a teacher rather than enlist in the forces. While teaching in Kent his enthusiasm, especially in the field, inspired at least one of his students to pursue a career in botany.

In October 1958 Clive successfully applied for the job of Head of the Fern Section at the (then) British Museum (Natural History), holding this post until 1990 when the De-

partment of Botany was reorganised and he became the first Head of Collections. Not content just to help revitalise curation, he was the inspiration and driving force behind the creation of the Museum’s Wildlife Garden.

Clive followed the example of his predecessor, A.H.G. Alston, undertaking a number of long overseas collecting trips. Some of these were major scientific expeditions, which he had a talent for organising: New Guinea (six months, 1964–65), Indonesia (Java and Sulawesi in 1969–70), Trinidad (1963 and 1974), Sarawak (1976, 1978 and 1990) and Sabah (1980). He also collected in Costa



Clive Jermy
with *Dryopteris dilatata* in Cwm Woods,
Aberystwyth, May 2006

Rica, Australia and Kenya, Macaronesia and many European countries as well as, of course, in the UK. On many of his main trips he joined forces with Trevor Walker of Newcastle University, who became a close friend. On later trips to Borneo he collected with Josephine Camus. In Europe he often travelled with Ronnie Viane. Clive wanted to collect more specimens than had Alston; this he achieved, reaching over 23,000 numbers as compared to Alston's 17,000+!

On one of his early collecting trips with a Museum colleague Clive was busy with his machete. Then he stopped and began to gather the plant material into bags. "I thought you were just clearing a trail", his colleague reputedly commented. Clive's reputation for bulk collecting led to his presentation on retirement of (among other gifts) a miniature combine harvester! But he was an expert collector with a good eye and his excellent herbarium collections (predominantly of ferns, but also of most other plant groups), with duplicates distributed to other institutions, will remain a lasting tribute to him. Spores and sporelings were also collected, for cultivation either at Newcastle, or at RBG Kew where they were expertly grown on by Bert Bruty and later John Woodhams, providing a rich resource for others.

In 1980 the Royal Geographical Society awarded Clive their prestigious Murchison Award for advancement of geographical science. This was in recognition of his key role as Scientific Co-ordinator of the RGS expedition to Gunung Mulu, Sarawak in 1977-78 and publication of a successful Management Plan for the National Park; he was also the first honorary head of the RGS's Expedition Advisory Centre, a trustee of the RGS Young Explorers Trust and had long been involved with the British Schools Exploration Society and the Brathay Exploration group.

News of Clive's death sparked a flow of tributes from around the world. A common thread reinforced the fact that one of Clive's most important roles was to inspire and encourage others, be they students starting out on their careers or more established scientists. He invited many pteridologists to London where he and his wife Alma welcomed them to their home in Otford, Kent, and many life-long friendships were made. In between the science he fitted in fern garden tours with visiting botanists such as Bert Hennipman and Chris Haufler.

Clive was involved with a wide range of pteridological projects. He worked with Mary Gibby and Christopher



Clive Jermy

with Mary Gibby & Alison Paul (*standing*),
Josephine Camus & Anne Sleep (*seated*) in the
NHM Fern Herbarium, October 1979

Fraser-Jenkins on *Dryopteris* in Europe and Macaronesia, and with Hugh Corley particularly on the male fern group. He retained an interest in the *Dryopteris affinis* complex and in the 1990s he set up 'Affinis watch' with Anthony Pigott, encouraging people to send in specimens for study. He was particularly interested in *Isoetes* – Clive could often be seen wading into ponds and lakes, and he notably collaborated with Ted Schelpe on the African species and Carl Taylor on those in Europe and North America. Clive set up a survey of *Pilularia* in Britain and Ireland, with over 70 people contributing between 1985 and 1987. The data was transcribed and published by Plantlife in 2000. He investigated *Diphasiastrum* with Ilka Kukkonen and *Huperzia* was also of interest; he collected many specimens of the latter, predicting that in the UK there was more than one taxon.

Clive joined the BPS in 1959 and was largely responsible for raising the Society's profile across the world and increasing the scientific membership. He took over Editorship of *The British Fern Gazette* the year he joined, and turned it into a respected scientific journal. He was Editor/Senior Editor until 1975 when he changed the title to the *Fern Gazette*, a decision he later regretted, continuing in a support role until 1983. He also edited the BPS Special Publication Series from 1984 until 2002. He was President from 1982 to 1985, a Vice-President from 1987 to 1993, was awarded the Stansfield Medal in 1991 and made an Honorary Member in 2002. He encouraged Patrick Acock and Paul Ripley to start the South-East Regional Group in 1984. Clive was also a keen fern grower and as such was able to relate to BPS members whatever their interest in ferns.

Clive organised many BPS field meetings in various parts of England, Scotland, Wales and Ireland, including one in 1997 to his native Norfolk, where his local knowledge and contacts ensured an excellent meeting. He especially loved visiting Wales and Scotland. Clive was like a mountain goat and also had great stamina; one often had to remind him that whilst *he* might not require lunch or a break, the rest of us needed to stop for sustenance!

Clive was on the organising committee of several of the Society's international fern conferences and senior editor of the resultant Proceedings: *The Phylogeny and classification of the ferns* (held jointly with the Linnean Society in 1972), the BPS Centenary symposium: *Cultivation and propagation of pteridophytes* (1991), and he co-ordinated the scientific programme of the BPS 2001 symposium: *Fern flora worldwide – threats and responses*.

Having sent out questionnaires to the world's pteridologists through the BPS and the American Fern Society, in 1973 Clive published with Joyce Holman *An international directory of Pteridologists*, a forerunner of the IAP's *Annual Review of Pteridological Research*. Clive started the International Association of Pteridologists in 1981, under the umbrella of the International Association of Plant Taxonomists. He was Chairman (1981-1987) and edited its Newsletter from 1986 to 1992. He was also one of

the handful of friends who set up the Group of European Pteridologists in 1992.

Over the course of his career, Clive's publications were numerous, often resulting from his many collaborations, and covered a wide range of topics. His first significant fern publication was in the 1959 *Fern Gazette* 9(1): *A preliminary census list of British pteridophytes*. In the early 1970s he instigated a joint project between the BPS and the BSBI to produce the 1978 *Atlas of ferns of the British Isles*. The 1975 publication (with Jim Crabbe and John Mickel) *A new generic sequence for the pteridophyte herbarium* has been used in many of the world's herbaria. But perhaps best known to current BPS members is his joint work with Josephine Camus, *The illustrated field guide to ferns and allied plants of the British Isles* (1991), which developed from the very successful 1987 *BM fern crib*. It is planned to publish a list of Clive's publications in *The Fern Gazette*.

Clive was an excellent field botanist and was heavily involved in the BM Botany Department's project on the isle of Mull; this programme of fieldwork through the late 1960s culminated in publication of the *Flora of Mull* in 1978, which he edited with Jim Crabbe.

Clive joined the Botanical Society of the British Isles in 1950, serving on the Council and Publications and Conservation Committees. He was a Vice-President (1991-1995) and an Honorary Member from 1997. He instigated the series of BSBI Handbooks, writing the first one, *Sedges of the British Isles* with T.G. Tutin from Leicester, published in 1968. Second and third (considerably expanded) collaborative editions were published in 1983 and 2007.

Clive was involved in plant conservation for much of his life, having a long association with the Norfolk Naturalists Trust and Kent Trust for Nature Conservation. His broad botanical and ecological knowledge enabled him to prepare management plans of assorted habitats from lowland English fens to South-East Asian Tropical rain forests and he tutored on training courses on plant identification, ecology and impact assessment. He was at the heart of the group that formed the Conservation Association of Botanical Societies (CABS) in 1987, from which grew Plantlife, founded in 1989. He was a committee member of the Fauna and Flora Preservation Society, and co-chair with David Given of the IUCN Species Survival Commission Pteridophyte Specialist Group.

In 1992 Clive retired but continued to visit the NHM as a Scientific Associate when he could, though he had little time after moving to Herefordshire on his marriage to Valerie in 1999, two years after Alma's sad death. In December 2009 he suffered a severe stroke, a particularly cruel blow for someone known for his boundless energy. Following Valerie's unexpected death, Clive moved to a care home near Oswestry, close to his son Stuart and family. Although conversation was difficult, he still remembered people, places and ferns and he seemed content. He died peacefully on 25th July and was buried in Kent with Alma. Friends and

family gathered on 12th August for a memorial thanksgiving service near Oswestry, the church bedecked with ferns and wild flowers. Our condolences go to Clive's daughter Nerida, Stuart and Liz and his grandchildren Rafael and Celeste.

Alison Paul

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COVER CAPTION: Proximal view of a megaspore of *Selaginella tenuissima* Fée, which is endemic to Brazil and recorded for the states of Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul (SE-Brazil, and S-Brazil). It grows in sandy humus, on mesic clay, and rocks.